STUDIES ON PHYTOPHTHORA CINNAMOMI RANDS

by

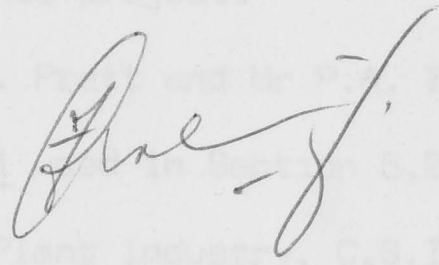
FRANS ARENTZ

Thesis submitted for the degree of Doctor of Philosophy
at the Australian National University

May, 1974

ORIGINALITY OF THESIS

Except where acknowledged this thesis is my own original work.



ACKNOWLEDGEMENTS

This work was financed by a Commonwealth Postgraduate Award.

I wish to thank Professor J.D. Ovington and the Department of Forestry, Australian National University, for providing the opportunity and facilities for carrying out this project.

I am indebted to Dr B.H. Pratt and Mr P.A. Taylor for the supply of cultures of *P. cinnamomi* used in Section 5.2.7. My thanks to Dr C.J. Shepherd, Division of Plant Industry, C.S.I.R.O., for helpful discussions throughout the period of the study, and Dr D.M. Griffin, Department of Forestry, A.N.U., for guidance and discussions during the final stages of the project.

I would like to acknowledge the assistance given by various people, notably Mr F. Darlington who carried out all nutrient analyses, the late Mr G.A. McIntyre who assisted with design of the field surveys, Mr R.I. Forrester, Division of Mathematical Statistics, C.S.I.R.O., who helped with statistical computations and Dr M.T. Tanton who supplied some of the computer programs used in the statistical analyses.

I would also like to acknowledge Dr W.A. Heather and Dr B.H. Pratt, Department of Forestry, A.N.U., for guidance and supervision throughout this project.

Finally I would like to thank Mrs E. Robinson for the typing of this thesis.

TABLE OF CONTENTS

LIST OF TABLES

Page No.

LIST OF FIGURES

Page No.

ABSTRACT

In Part A of this dissertation the results of a survey to examine the distribution of *P. cinnamomi* in forest soil at Termeil near Batemans Bay, New South Wales, are presented and discussed. The distribution of the fungus was determined from a field survey in which sampling sites were located at random from a square grid superimposed on a large-scale map of the area. The fungus was recovered in all topographic situations independent of aspect, overstorey *Eucalyptus* spp. association, soil pH and distance from access roads. The recovery of *P. cinnamomi* appeared to be inversely related to soil moisture levels which had been rated subjectively on a scale of 1-3.

A further comparison of sampling techniques suggested that the distribution of the fungus in soil was discontinuous, both in space and time. The widespread distribution of *P. cinnamomi* in the area, the absence of widespread manifest disease and the present distribution pattern suggested that an equilibrium had been established between *P. cinnamomi*, potential hosts and environment. Intensive sampling of a two-hectare site extending over a topographic gradient indicated that there was a higher frequency of microhabitats favourable for survival of *P. cinnamomi* in the gully compared with the upper slopes.

The radial growth rates of 97 isolates of *P. cinnamomi* obtained during the field surveys fell within the range previously recorded for other Australian isolates and it was suggested that the 97 isolates came from a single population. Examination of the mating type of 138 isolates of *P. cinnamomi* obtained from the study area indicated that 5 were A1 and the remaining 133 were A2.

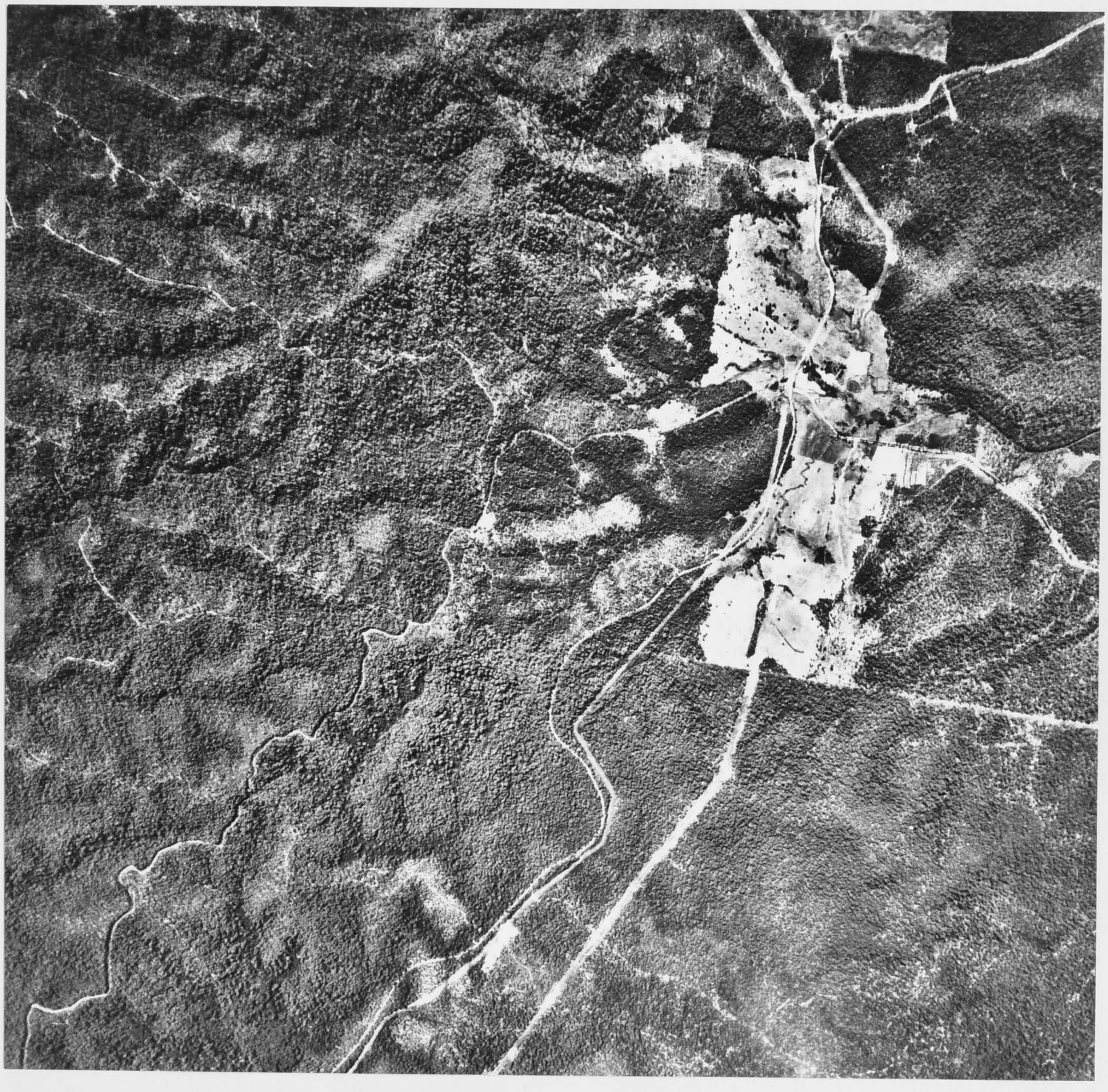
It was concluded that determination of the micro-environmental factors associated with the activity of P. cinnamomi in soil using field survey techniques was hampered by limitations in the sampling techniques. It was further suggested that extrapolation of the determined distributions of P. cinnamomi in space and time was invalid unless these factors were known.

In Part B the effects of calcium and other cations on the colonization of blue lupin roots by P. cinnamomi was examined. An increase in nutrient availability from 0.0003 M to 0.01 M significantly reduced the colonization of blue lupin roots by P. cinnamomi in a nutrient culture system. However there was no significant reduction in lesion length with comparable variations in magnesium concentration. Similarly changes in pH of the nutrient medium did not significantly affect lesion length. When the potassium level of the basic nutrient solution was raised to 0.1 M there was a significant increase in lesion length. Similar results were obtained in nutrient solutions with an aluminium level of 0.001 M.

Comparison of colonization of blue lupin roots by A1 and A2 mating types indicated no difference in lesion length in 0.0003 M calcium whereas in 0.004 M calcium the A1 isolates colonized significantly less root tissue than the A2 isolates.

It was proposed that mechanisms of reduced pectin availability, resulting from bonding of calcium with polygalacturonic acid units in the middle lamellar zone, and reduced membrane permeability with increased calcium availability were involved in the reduction of colonization of lupin roots by P. cinnamomi.

Additional experiments to examine further these mechanisms, both in blue lupins and Eucalyptus spp., were outlined and discussed. An approach to determine the effectiveness of calcium in reducing the incidence of disease associated with P. cinnamomi in plant species in a field situation was discussed.



Aerial photograph of Termeil and surrounding forests (see Chapter 2)

INTRODUCTION

The soil-borne fungus Phytophthora cinnamomi Rands has a world-wide distribution (Crandall & Gravatt, 1967; C.M.I. Map 302, ed. 3, 1968) and has been associated with the deaths of a wide range of plant species (Crandall & Gravatt, 1967; Zentmyer & Thorn, 1967; Newhook & Podger, 1972). In Australia the pathogen has been recovered in every State in association with root rots of horticultural plants, exotic conifers or native forests (Pratt et al., 1973).

The first association of P. cinnamomi with disease in Australian native understorey shrubs was made in 1956 (Fraser, 1956), but the fungus was not regarded as a serious pathogen of native forests until 1965 when it was isolated from areas of dying Eucalyptus marginata Donn. ex. Sm. in Western Australia (Podger et al., 1965). Subsequent reports have associated P. cinnamomi with deaths in native vegetation in a wide range of environments (Newhook & Podger, 1972; Pratt & Heather, 1973 a) although the fungus has been isolated also from areas showing no obvious disease (Newhook & Podger, 1972; Pratt & Heather, 1973 a).

An understanding of these root-rots, and the development of control measures, has been hampered by a lack of definitive information on the factors affecting distribution of the organism within different types of forest communities, and on environmental factors which affect the host-pathogen interaction.

To overcome some of these deficiencies a detailed study has been made of P. cinnamomi in a mixed hardwood eucalypt forest in southern coastal New South Wales, and the results are presented herein.

Field studies were carried out to determine the influence of environmental factors on the distribution of the fungus and on the occurrence of disease in the study area. Associated laboratory studies were made to determine the influence of mineral nutrition on infection and subsequent development of the fungus within the host plant. Additional studies were made to determine the occurrence of different mating strains of the fungus.

CHAPTER 1

LITERATURE REVIEW

1.1 DISEASE

Disease resulting in the death of understorey and overstorey vegetation of eucalypt forests has been reported in all Australian States and *P. cinnamomi* has been recovered from many of the affected areas (Podger, Doepel & Zentmyer, 1965; Podger & Ashton, 1970; Weste & Taylor, 1971; Marks *et al.*, 1972; Pratt & Heather, 1973 a). The pattern of symptom development is similar in most areas with chlorosis and wilting appearing initially in the understorey vegetation (Weste & Taylor, 1971; Marks *et al.*, 1972; Podger, 1972; Pratt & Heather, 1973 a). Further disease symptoms include premature defoliation, microphylly, epicormic shooting, stunting and loss of fine branches (Podger, 1972; Pratt & Heather, 1973 a). In both understorey and overstorey vegetation death can occur suddenly within a few days of the initial onset of symptoms, although in overstorey trees deterioration of the crown can occur over a number of years (Podger, 1972; Pratt & Heather, 1973 a).

The significance of *P. cinnamomi* in disease in forest plants is controversial. Adverse soil environment was claimed to be the primary cause of high tree mortality in a plantation of *Pinus radiata* D. Don, with *P. cinnamomi* playing only a minor role (Jehne, 1971). However, in New Zealand, Newhook (1959) concluded that deaths in shelterbelts of *P. radiata* could not be attributed to an adverse soil

environment alone and suggested that P. cinnamomi contributed significantly to disease. Other workers have attributed deaths in native forests directly to P. cinnamomi, with less emphasis on the importance of other organisms or environmental factors in the development of disease (Podger & Ashton, 1970; Weste & Taylor, 1971; Podger, 1972; Marks et al., 1972). Pratt & Heather (1973 a), in emphasizing the importance of environment in the host-pathogen interaction, concluded that disease caused by P. cinnamomi could not always be distinguished from that caused by other factors and therefore referred to the disease "as one associated with the presence of P. cinnamomi". In addition, potentially pathogenic Phytophthora and Pythium species have been isolated from native forest areas and it was suggested that disease normally associated with P. cinnamomi could also be induced by these organisms (Pratt & Heather, 1973 b).

1.2 HOST RANGE

P. cinnamomi has been isolated from the roots and root zones of many species, particularly from the families Myrtaceae, Proteaceae, Leguminosae and Epacridaceae (Zentmyer & Thorn, 1967; Newhook & Podger, 1972; Pratt & Heather, 1973 a). Within the genus Eucalyptus L'Herit., species of the sub-genus *Monocalyptus appeared to be most susceptible to disease whereas species of the sub-genera Blakella, Corymbia, Eudesmia, Idiogenes and Symphyomyrtus appeared to be resistant or

---

* Based on "A Classification of the Eucalypts" by L.D. Pryor & L.A.S. Johnson, Australian National University, 1971.

tolerant (Newhook & Podger, 1972; Pratt & Heather, 1973 a). The sub-genus Monocalyptus contains many commercially important timber species susceptible to P. cinnamomi root rot, including Eucalyptus sieberi L. Johnson, E. pilularis Sm., E. obliqua L'Herit., and E. regnans F. Muell. in south-eastern Australia, and E. marginata in Western Australia (Pratt & Heather, 1973 a).

1.3 ENVIRONMENTAL FACTORS ASSOCIATED WITH DISEASE

In Western Australia and some areas in Victoria a constant association between disease and P. cinnamomi was demonstrated, and the fungus was not detected in apparently healthy forest stands (Weste & Taylor, 1971; Podger, 1972; Marks et al., 1972). However, in other areas in eastern and southern Australia P. cinnamomi was recovered from sites which showed no manifest disease (Pratt & Heather, 1973 a). Field inoculation of E. marginata stands with P. cinnamomi was followed by the development of manifest disease in the vegetation and this was attributed to the fungus (Podger, 1972). However, the mechanisms by which environmental factors influence the interaction of host and pathogen in Phytophthora root rot disease are not fully understood. Several factors may interact, making it difficult to define the cause of disease (Newhook & Podger, 1972; Pratt & Heather, 1973 a). A number of environmental factors, associated with the occurrence of disease in areas from which P. cinnamomi has been recovered, have been examined and major findings of these studies included:-

1. Disease occurred most frequently on soils with impeded drainage (Weste & Taylor, 1971; Pratt & Heather, 1971, pers. comm.; Podger, 1972; Marks et al., 1972), although the disease occurred also on deep well-drained gravels (Podger, 1972; Newhook & Podger, 1972).

2. Manifest disease was most severe following periods of exceptionally high rainfall (Weste & Taylor, 1971; Marks et al., 1972; Pratt et al., 1972 a; Newhook & Podger, 1972), and it has been suggested that free soil moisture was required for the dispersal of zoospores and infection of plant roots (Newhook, 1959).

3. Pronounced disease was closely associated with road construction (Weste & Taylor, 1971; Podger, 1972; Marks et al., 1972; Pratt et al., 1973). It has been suggested that this was related to the distribution of the inoculum (Podger & Ashton, 1970; Weste & Taylor, 1971; Podger, 1972; Pratt et al., 1973) as well as an increase in the soil moisture status resulting in the development of environments more suitable for fungal activity (Pratt et al., 1973).

4. Disease was more frequent along drainage lines and gullies than on upper slopes and ridges, although it appeared to be independent of aspect and slope (Podger, 1972).

5. Manifest disease occurred often on soils with low cation - exchange capacities (Podger & Ashton, 1970; Pratt & Heather, 1971, pers. comm.; Podger, 1972). In the United States of America the incidence of littleleaf disease in Pinus echinata Mill. and P. taeda L., which has been associated with P. cinnamomi, was reduced with applications of inorganic nitrogen fertilizer (Campbell & Copeland, 1954).

Similarly in New Zealand, applications of superphosphate reduced the incidence of littleleaf disease in P. radiata (Newhook & Podger, 1972). However, in Western Australia, application of fertilizers (form not specified) apparently did not reduce the incidence of manifest disease in E. marginata forests (Newhook & Podger, 1972) although the fertilizers were added before P. cinnamomi had been recognized as a factor in the occurrence of disease (c.f. Newhook & Podger, 1972).

1.4 THE EFFECT OF ENVIRONMENTAL FACTORS ON P. CINNAMOMI AND SOME FUNDAMENTAL CHARACTERISTICS OF THE ORGANISM

The life cycle of P. cinnamomi is illustrated in Fig. 1.1. The following factors have been shown to affect the growth and reproduction of P. cinnamomi in vitro:-

1. Cardinal temperatures for radial growth of hyphae on 2% cornmeal agar were between 5°C and 10°C minima, 30°C and 35°C maxima and with an optimum between 22.5°C and 30°C (Shepherd & Pratt, 1974). However the cardinal temperatures varied considerably between isolates and with the type of medium used (Shepherd & Pratt, 1974).

2. The optimum pH range for growth on agar with an amino nitrogen source was 4.8 - 7.5 (C.J. Shepherd, pers. comm.).

3. There was little change in the linear growth rate of hyphae for a reduction in oxygen partial pressure from 0.21 atm. to 0.002 atm. (Griffin, 1972).

4. Optimum growth of hyphae occurred at an osmotic potential of -10 to -15 bars(Sommers et al., 1970). Reduced growth was evident at a matric potential of -10 bars which was possibly the result of

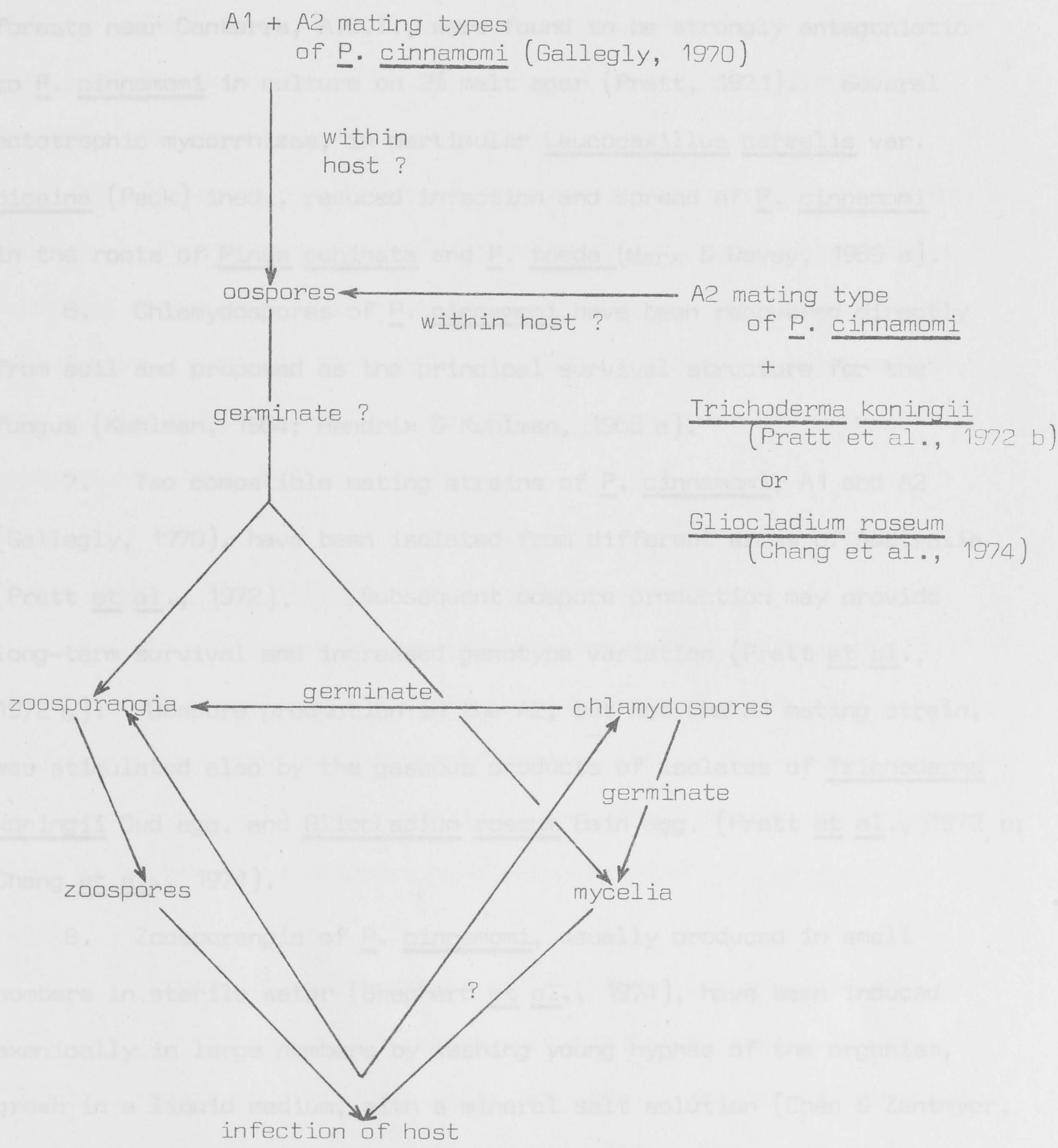


FIG 1.1 Life cycle of P. cinnamomi

restricted transport of nutrients to, or carriage of metabolic by-products away from, the fungal hyphae (Adebayo & Harris, 1971).

5. Isolates of ten basidiomycete genera obtained from eucalypt forests near Canberra, A.C.T., were found to be strongly antagonistic to P. cinnamomi in culture on 2% malt agar (Pratt, 1971). Several ectotrophic mycorrhizae, in particular Leucopaxillus cerealis var. piceina (Peck) ined., reduced infection and spread of P. cinnamomi in the roots of Pinus echinata and P. taeda (Marx & Davey, 1969 a).

6. Chlamydospores of P. cinnamomi have been recovered directly from soil and proposed as the principal survival structure for the fungus (Kuhlman, 1964; Hendrix & Kuhlman, 1965 a).

7. Two compatible mating strains of P. cinnamomi, A1 and A2 (Gallegly, 1970), have been isolated from different areas of Australia (Pratt et al., 1972). Subsequent oospore production may provide long-term survival and increased genotype variation (Pratt et al., 1972 b). Oospore production in the A2, but not the A1 mating strain, was stimulated also by the gaseous products of isolates of Trichoderma koningii Oud agg. and Gliocladium roseum Bain agg. (Pratt et al., 1972 b; Chang et al., 1974).

8. Zoosporangia of P. cinnamomi, usually produced in small numbers in sterile water (Shepherd et al., 1974), have been induced axenically in large numbers by washing young hyphae of the organism, grown in a liquid medium, with a mineral salt solution (Chen & Zentmyer, 1970).

9. Several bacterial species belonging to the Pseudomonadaceae (Marx & Haasis, 1965) as well as Chromobacterium vidaceum (Schroeter) Bergonzini, isolated from a soil in South Australia (Zentmyer, 1965),

have been shown to induce abundant zoosporangial production in P. cinnamomi in vitro. Mechanisms of direct stimulation or removal of inhibitors have been proposed for this induction (Marx & Bryan, 1969). Other stimuli, such as nutrient depletion (Chen & Zentmyer, 1970) and the indirect action of bacterial metabolites (Chee & Newhook, 1965) have also been implicated.

10. A chemotactic response by zoospores to the roots of avocado seedlings (Persea americana Mill.) and other hosts has been observed, with the encysted zoospores accumulating mainly in the zone of root elongation (Zentmyer, 1961; Hickman, 1970). However, zoospores were not attracted to the roots of several Pinus species with fully formed mycorrhizae (Marx & Davey, 1969 b).

1.5 FUNGAL POPULATIONS

An assessment of the influence of certain environmental factors on the activity and survival of a pathogen in soil presupposes a technique to accurately measure the population density of the pathogen in the soil (Baker, 1970). Several techniques have been used to estimate the relative population level of P. cinnamomi in soil.

Kuhlman (1964) attempted to relate percentage infection by P. cinnamomi of apple baits, which were exposed to a soil, to the relative abundance of the fungus in the soil. However soil samples containing the fungus do not necessarily infect the apple bait, thus resulting in an unknown margin of error (Newhook, 1959). The percentage infection of lupin radicles by P. cinnamomi has been

suggested as an alternative method (Pratt & Heather, 1972) although it may be subject to the same error as apple baits.

Reduction of inoculum levels of the fungus in the soil by serial dilution of infested soil with a sterilized soil, and subsequent baiting to provide an estimate of disease potential was tested by Tsao (1960).

Techniques dependent on the indirect use of baits do not necessarily show quantitative differences in absolute numbers of propagules and therefore may not be suitable for estimates of population size. This deficiency was overcome, in part, by the development of an agar medium, selective for Phytophthora spp., which enabled the use of direct plating of soil particles and soil dilution plating (Hendrix & Kuhlman, 1965b) to give estimates of the numbers of propagules able to germinate and develop under the conditions of the experiment. However, the technique gives no indication of the type of propagule from which colonies arise nor distinguish between propagules with different capacities to germinate. In addition all of the techniques, whether by baiting or soil plating, fail to measure the state of activity of the fungus at the time of sampling (Harley, 1972).

1.6 SURVEY TECHNIQUES

The following sampling techniques have been used to examine the distribution of P. cinnamomi in forest soils, and of disease associated with the fungus:-

1. Soil and root samples were collected from sites selected subjectively on the basis of presence of obvious disease in vegetation.

These were then compared with similar samples from sites in adjacent unaffected forest stands (Podger, 1972).

2. Soil and root samples were collected along transect lines extending from apparently unaffected areas into adjacent areas with dead or dying vegetation (Weste & Taylor, 1971; Marks et et al., 1972).

3. Soil samples were collected at the base of diseased and apparently healthy plants along transect lines traversing diseased and apparently healthy areas, different forest types, and different topographical sites (Pratt & Heather, 1973 a).

Composite (Podger, 1972, Marks et al., 1973) as well as single soil samples (Weste & Taylor, 1971; Marks et al., 1972; Pratt & Heather, 1973a) were examined in the laboratory for the presence of P. cinnamomi by baiting with blue lupins (Lupinus angustifolius L.) (Weste & Taylor, 1971; Podger, 1972; Pratt & Heather, 1972) or with cotyledons of Eucalyptus sieberi (Marks et al., 1973). In addition host root material was plated directly onto 3P agar of Eckert & Tsao (1962) (Podger et al., 1965; Weste & Taylor, 1971; Podger, 1972).

In all of the survey techniques described there was some bias in the initial selection of sampling sites. This bias has prevented meaningful statistical comparisons of environmental parameters associated with the distribution of P. cinnamomi. In a field survey, reported in Part A of this dissertation, this bias was minimised by the random selection of sampling sites using a two-dimensional grid superimposed on a large scale map of the study area. At each site data were collected on topographic position, aspect, overstorey species association, distance from roads, soil pH, relative soil moisture and

amount of ground cover as well as the presence of P. cinnamomi. This sampling technique ensured the inclusion of a large number of replicate sites which could be compared statistically.

It has been proposed that the increase in host resistance to disease associated with P. cinnamomi, which occurred with applications of fertilizers, was a result of reduced infection of roots by the pathogen, as well as enhanced host vigour (Newhook & Podger, 1972). In Part B of this thesis the effects of calcium and other cations on the infection and colonization of lupin roots by P. cinnamomi is examined, in order to determine whether changes in resistance are also associated with changes in root physiology.

PART A

CHAPTER 2

DISTRIBUTION OF P. CINNAMOMI IN FOREST SOIL IN THE BATEMANS BAY AREA OF NEW SOUTH WALES

2.1 INTRODUCTION

P. cinnamomi was recovered from forest soils on the south coast of New South Wales during a survey to determine the broad distribution of the fungus in eastern Australia (Pratt *et al.*, 1973). In the present study an attempt was made to determine, in greater detail, the distribution and factors affecting the distribution of *P. cinnamomi* in forest soil in the Batemans Bay area of the south coast by:-

1. Sampling sites selected at random for *P. cinnamomi* using a bulked soil core technique;
2. Resampling sites more intensively to determine whether *P. cinnamomi* could be isolated from sites where it was not recovered in the initial survey;
3. Intensive sampling of a single locality to determine the distribution of *P. cinnamomi* over a topographic range;
4. Determining the mean radial growth rate and mating type of each isolate of *P. cinnamomi*.

2.2 DESCRIPTION OF STUDY AREA

2.2.1 Location

The study area was located in eucalypt forest surrounding Termeil, approximately 35 km north of Batemans Bay (Fig. 2.1).

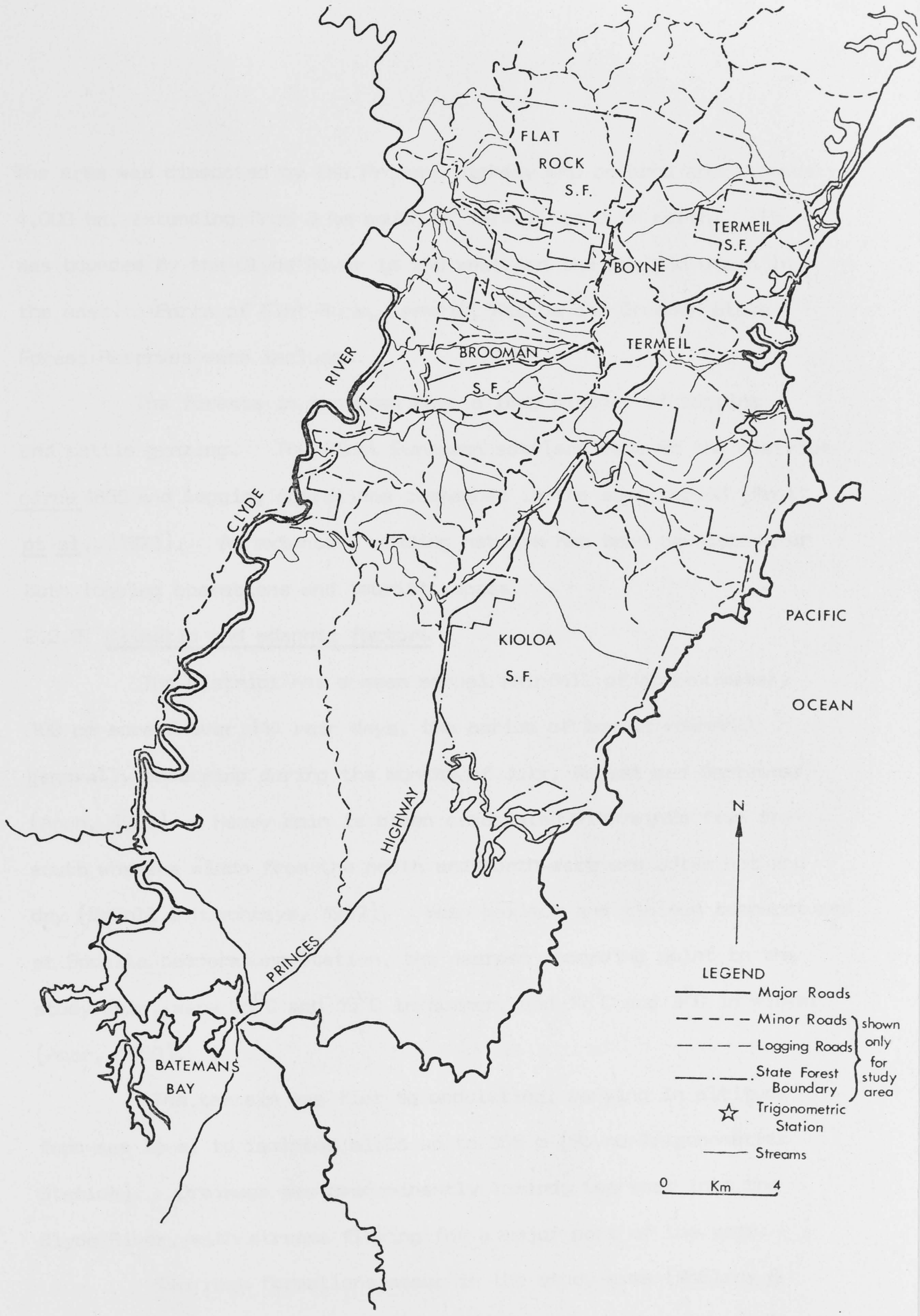


FIG 2.1 Location of study area

The area was dissected by the Princes Highway and covered approximately 4,000 ha, extending from 3 km south of Termeil to 3 km north. It was bounded by the Clyde River in the west and the Pacific Ocean in the east. Parts of Flat Rock, Termeil, Kioloa and Brooman State Forest Reserves were included.

The forests in the area have a long history of logging and cattle grazing. The first European settlers came to the district circa 1850 and logging operations commenced in the same period (Pratt et al., 1973). An extensive roading network has been developed for both logging operations and tourist access.

2.2.2 Climatic and edaphic factors

The district has a mean annual rainfall of approximately 100 cm spread over 114 rain days, the period of lowest rainfall generally occurring during the months of July, August and September (Anon, 1966). Heavy rain is often associated with winds from the south whereas winds from the north and north-west are often hot and dry (McColl & Humphreys, 1967). Mean maximum and minimum temperatures at Bodalla temperature station, the nearest recording point to the study area, were 25°C and 15°C in summer, and 17°C and 3°C in winter (Anon, 1969).

The terrain was flat to undulating, varying in altitude from sea level to isolated hills up to 356 m (Boyne Trigonometric Station). Drainage was predominantly towards the west into the Clyde River, with streams flowing for a major part of the year.

Two rock formations occur in the study area (McElroy & Rose, 1962). The Conjola Formation of Permian age extends east

from Boyne Trigonometric station to the coast (Fig. 2.1) and consists of conglomerate, sandstone and siltstone. The second formation, of metamorphosed sediments of Ordovician age, extends west of the Conjola Formation. It consists mainly of micaceous siltstone, mudstone and quartzite.

Two soil types predominate in the study area (Northcote, 1966); brown friable earths (type Gn 3.2) associated with hard acidic yellow mottled soils (types Dy 3.21 and Dy 3.41) on the moderate to steep slopes in the west of the study area, and duplex yellow soils (types Dy 2.41 and Dy 3.41) in the east. Dunes of leached sands (type Uc 2.2) occur in low-lying situations along the coast.

Soils on ridges tended to be shallow with a high sand content and there was a gradation to gullies of deep silty-clay loams with a high organic content. These gully soils had a high ratio of capillary to non-capillary porosity, indicating slow movement of soil moisture and poor soil aeration, whereas soils on ridges had high soil moisture flow rates (McColl, 1969; Furrer, 1971).

2.2.3 Vegetation

McColl and Humphreys (1967) recognized four main *Eucalyptus* species "associations" which tended to be related to topography:-

1. *Eucalyptus gummifera* (Gaertn.) Hochr. dry sclerophyll association found on ridges on shallow red to yellow podzolic soils (type Dy 3.41) derived from shale and quartzite parent material. Codominant species included *Angophora intermedia* D.C., *E. globoidea* Blakely, *E. pilularis*, *E. piperita* Sm., *E. sieberi* and *Casuarina littoralis* Salisb.

2. E. maculata Hook - E. paniculata Sm. dry sclerophyll association found on moderate slopes of brown to yellow podzolic soils (type Gn 3.2) derived from shale or micaceous sandstone parent material. Codominant species included E. globoidea, E. muellerana Howitt., E. pilularis, E. piperita and E. sieberi.

3. E. maculata - E. pilularis dry sclerophyll association found on slopes in a discontinuous manner with the E. maculata - E. paniculata association, although codominant species were common to both associations. Furrer (1971) combined both associations into a general E. maculata association, as will be done in this study. The E. maculata - E. pilularis association was generally found on soils (type Gn 3.2) derived from slate or schist parent material.

4. E. saligna Sm. wet sclerophyll association found in gullies on kraznozems (type Gn 3.12). Rainforest species were present forming a large component of the understorey.

Narrow ecotones existed between all of the species associations except between the E. gummifera and E. saligna associations. The separation of E. gummifera and E. maculata into discrete stands could not be associated with any marked edaphic discontinuity, although it did occur across gradients of physical soil properties (McColl & Humphreys, 1967) and of increasing soil nutrient availability from ridge to gully sites (McColl, 1969).

McColl (1965) listed understorey species which were commonly found in the eucalypt associations. These include:-

1. E. gummifera association

Lepidosperma filiforme Labill., Pteridium aquilinum (L.) Kuhn, Acacia obtusifolia A. Cunn., Acacia decurrens (Wendl.) Willd.,

Acacia maidenii F. Muell., Lomandra spp., Leucopogon lanceolatus R.Br., Banksia spinulosa Sm., Bossiaea microphylla Sm.

2. E. maculata association

Poa spp., Doodia aspera R.Br., Tylophora barbata R.Br., Acacia longissima Wendl., Macrozamia communis L. Johnson, Pteridium aquilinum, Lepidosperma filiforme, Blechnum cartilagineum Sw., Acacia obtusifolia, Elaeocarpus cyaneus Ait., Cissus antarcticus Vent., Acacia irrorata Sieb. ex Spreng.

3. E. saligna association

Pteridium aquilinum, Macrozamia communis, Indigofera australis Willd., Smilax autralis R.Br., Hibbertia dentata R.Br. ex DC., Tristania spp., Blechnum cartilagineum, Rhodamnia trinervia (Sm.) Blume, Acmena smithii (Poir.) Merrill et Perry, Goodenia ovata Sm., Acacia decurrens, Backhousia myrtifolia Hook. et Harv., Livistona australis (R.Br.) Mart.

During the field surveys, reported herein, other species of the family Papilionaceae were also encountered; in particular Oxylobium ilicifolium (Andr.) Domin. and Hardenbergia violacea (Schneev.) Stearn.

2.3 INITIAL FIELD SURVEY

2.3.1 Experimental

A major field survey, carried out in June-September, 1971 to determine the distribution of P. cinnamomi and environmental factors associated with the occurrence of the fungus, was designed in consultation with Mr G.A. McIntyre, Division of Mathematical Statistics, C.S.I.R.O., Canberra. A systematically numbered square grid was superimposed on a 1 : 31680 scale map of the study area and one hundred sampling sites

selected using a table of random numbers (Cambridge Elementary Statistical Tables, 1952). The sites were located in the field using a map, compass bearings and topographic features and then categorised initially on the basis of topography (i.e. ridge, midslope or gully) and Eucalyptus species association. Environmental parameters such as aspect, distance from roads and density of ground cover were also recorded. Relative soil moisture levels were measured subjectively on a basis of moist, intermediate and dry. Soil pH measurements were made in the laboratory.

At each site five 100 gm cores of soil and root material, taken from the upper 15 cm of soil at 0.5 m intervals along the contour, were bulked and mixed. A subsample of approximately 150 gm was taken from the bulked cores and returned to the laboratory in a sealed aluminium canister. Two such samples were collected at each site. In the laboratory the soil samples were examined for the presence of P. cinnamomi within three days of collection using the blue lupin baiting technique of Chee and Newhook (1965) as modified by Pratt and Heather (1972). Roots of all lupins were plated on water/50 ppm streptomycin agar five days after commencement of baiting to confirm the presence of P. cinnamomi. Phytophthora species growing from the lupin roots into the agar were isolated onto 2% malt agar and maintained in stock culture at 8°C for further identification and study. The identification of the isolates was confirmed by growing them on 2% corn meal agar and comparing morphological features with the key of Waterhouse (1963). The mechanism of zoospore release and the size and shape of zoosporangia were examined by placing discs of the isolates, which had been cultured on 2% V8 agar, into a soil extract to induce zoosporangia formation.

Eucalyptus maculata association -
Brooman State Forest Reserve

Eucalyptus maculata association - showing
understorey vegetation and ground cover

2.3.2 Results

The results of the field survey were tabulated using two environmental parameters at a time, each of which was sub-divided into component parts to form cells. Each sampling locality was placed into its representative cell and the number of sites in each cell from which P. cinnamomi was recovered was expressed as a proportion of the total number of sampling localities within that cell. Two statistical tests were carried out.

1. Chi-square analysis (Siegel, 1956) to determine whether the two variables used in each table were inter-related or independent of each other. Both the expected and observed frequencies of sites within each cell are shown in the tables.

2. Two-factor analysis of variance (G. McIntyre, pers. comm.) to determine whether the presence of P. cinnamomi was related to either of the two variables or to an interaction of the two variables. For the analyses the sites from which the fungus was recovered was expressed as a percentage of the total number of sites in each cell, and all the data were subjected to an arcsine transformation.

P. cinnamomi was recovered from seventy-three of the one hundred sites sampled. The absolute absence of the fungus in the remaining twenty-seven sites could not be demonstrated because of limitations in the sampling technique and in the sensitivity of the lupin baiting technique (Pratt & Heather, 1973a).

Only ninety-seven sites were included in the statistical analysis as three of the sites, located in stands of Eucalyptus botryoides - Casuarina glauca Sieb. ex Spreng on low lying sandy soil

(type Uc 2.2), were found to be atypical of the study area as a whole. *P. cinnamomi* was isolated from all three sites.

During the survey soil samples were bulked so as to increase the probability of detecting the presence of the fungus in each sample as well as reducing the number of soil samples which had to be collected at each site. *Phytophthora* and *Pythium* species in addition *P. cinnamomi* were recovered from the soil samples by the lupin baits. These included an isolate of *Phytophthora citricola* Sawada, an isolate of *P. drechsleri* Tucker and several isolates of *Phytophthora* and *Pythium* spp. which were not identified.

To determine whether *P. cinnamomi* could be recovered directly from roots direct plating of the roots of several understorey species was carried out. One isolate of *P. cinnamomi* was obtained from the roots of *Leucopogon lanceolatus* although few replicate platings were carried out.

Measurements were made of soil pH and mean readings of pH 5.44 ± 0.34 (ridge), pH 5.34 ± 0.35 (midslope) and pH 5.48 ± 0.37 (gully) were obtained, suggesting that there was no association between the measured soil pH values and topography. Similarly there was no association between soil pH and the recovery of *P. cinnamomi*.

TABLE 2.3.1 Sites positive for *P. cinnamomi* on basis of species association and topography

Eucalyptus spp. association	Topography			
	Ridge	Midslope	Gully	Total
E. gummifera	8/10 (7.7)[a]	10/14 (13.6)	1/1 (3.6)	19/25
E. maculata	15/19 (16.7)	23/32 (29.5)	2/3 (7.8)	40/54
E. saligna	0/1 (5.6)	5/7 (9.9)	6/10 (2.6)	11/18
Total	23/30	38/53	9/14	70/97

[a] Expected frequency (from chi-square analysis)

From Table 2.3.1 the species associations were found to be related to topography (X^2 = 6.87, d.f. = 2, p =< 0.05), the E. gummifera association being restricted to the ridges and upper slopes and the E. saligna association being confined to gullies and lower slopes (c.f. McColl, 1969). A two-factor analysis of variance indicated no association between the presence of P. cinnamomi and the two factors of species association and topography.

TABLE 2.3.2 Sites positive for P. cinnamomi on basis of distance from roads and topography

Distance from roads	Topography			
	Ridge	Midslope	Gully	Total
<20 metres	13/18 (9.9)[a]	11/12 (17.5)	2/2 (4.6)	26/32
>20 <100 metres	9/11 (10.5)	16/22 (18.6)	1/1 (4.9)	26/34
>100 metres	1/1 (9.6)	11/19 (16.9)	6/11 (4.5)	18/31
Total	23/30	38/53	9/14	70/97

[a] Expected frequency

From Table 2.3.2 the topographic location of the sampling sites was found to be related to distance from roads (X^2 = 14.66, d.f. = 2, p =< 0.001). This reflected the roading network of the study area, with the majority of the roads being restricted to ridge-tops. A two-factor analysis of variance indicated no association between the presence of P. cinnamomi and the two factors of distance from roads and topography.

TABLE 2.3.3 Sites positive for P. cinnamomi on basis of soil moisture levels and topography

Soil moisture level	Topography			
	Ridge	Midslope	Gully	Total
Dry	12/15 (10.8)[a]	18/19 (19.2)	1/1 (5.1)	31/35
Intermediate	8/11 (9.6)	8/15 (16.9)	3/5 (4.5)	19/31
Moist	3/4 (9.6)	12/19 (16.9)	5/8 (4.5)	20/31
Total	23/30	38/53	9/14	70/97

[a] Expected frequency

From Table 2.3.3 moisture was found to be related to topography (χ^2 = 11.55, d.f. = 4, p =<0.05), with a higher frequency of dry sites occurring on ridges and of moist sites in gullies compared with the expected frequencies. Similarly a two-factor analysis of variance indicated that the recovery of P. cinnamomi from dry soils was significantly higher (p = 0.05) than from soils with intermediate and high moisture levels.

TABLE 2.3.4 Sites positive for P. cinnamomi on basis of species association and soil moisture levels

Eucalyptus spp. association	Soil moisture level			
	Dry	Intermediate	Moist	Total
E. gummifera	11/14 (9.0)[a]	5/6 (8.0)	3/5 (8.0)	19/25
E. maculata	17/18 (19.5)	11/20 (17.2)	12/16 (17.2)	40/54
E. saligna	3/3 (6.5)	3/5 (5.8)	5/10 (5.8)	11/18
Total	31/35	19/31	20/31	70/97

[a] Expected frequency

From Table 2.3.4 the Eucalyptus spp. associations were found to be related to soil moisture levels (χ^2 = 10.09, d.f. = 4, p =< 0.05). There was a higher frequency of the E. gummifera associated on dry soils compared with moist soils. Similarly the E. saligna association

occurred predominantly on moist soils. However these correlations may be related to the correlation between species association and topography (Table 2.3.1) as well as between soil moisture levels and topography (Table 2.3.3). A two-factor analysis of variance indicated that the recovery of P. cinnamomi from dry soils was significantly higher ($p = 0.05$) than from soils with intermediate and high moisture levels.

TABLE 2.3.5 Sites positive for P. cinnamomi on basis of soil moisture levels and ground cover

Soil moisture level	Ground cover			
	>50% litter	>50% litter + grasses	<50% litter	Total
Dry	12/12 (14.8)[a]	4/6 (8.6)	15/17 (11.6)	31/35
Intermediate	8/15 (13.1)	5/6 (7.7)	6/10 (10.2)	19/31
Moist	8/14 (13/1)	9/12(7.7)	3/5 (10.2)	20/31
Total	28/41	18/24	24/32	70/97

[a] Expected frequency

From Table 2.3.5 soil moisture levels were found to be related to ground cover ($X^2 = 9.77$, d.f. = 4, $p < 0.05$). Dry soils were associated with a litter cover of <50% whereas moist soils were associated with a ground cover of >50% litter and grasses. A two-factor analysis of variance indicated that the recovery of P. cinnamomi from dry soils was significantly higher ($p = 0.05$) than from soils with intermediate and high moisture levels.

TABLE 2.3.6 Sites positive for P. cinnamomi on basis of soil moisture levels and aspect

Soil moisture level	Aspect				
	0^o-90^o	90^o-180^o	180^o-270^o	270^o-360^o	Total
Dry	13/14 (11.2)[a]	1/2 (5.8)	10/11 (10.1)	7/8 (8.0)	31/35
Intermediate	5/8 (9.9)	5/6 (5.1)	7/9 (8.9)	5/8 (7.0)	19/31
Moist	5/9 (9.9)	7/8 (5.1)	3/8 (8.9)	5/6 (7.0)	20/31
Total	23/31	10/16	20/28	17/22	70/97

[a] Expected frequency 0^o = North

From Table 2.3.6 soil moisture levels were found to be independent of aspect ($\chi^2 = 5.90$, d.f. = 6, n.s.). A two-factor analysis of variance indicated that the recovery of P. cinnamomi from dry soils was significantly higher ($p = 0.05$) than from soils with intermediate and high moisture levels.

Results of a further statistical analysis of environmental parameters which were not significantly associated with the distribution of P. cinnamomi in soil in the Batemans Bay area are shown in Appendix I.

2.4 RESAMPLING OF SOILS

2.4.1 Experimental

Ten of the sites sampled in the first survey (June-September, 1971) were resampled more intensively in November, 1972, to determine:-

1. Whether P. cinnamomi could be re-isolated from those sites in which the fungus was recovered during the first sampling.
2. Whether P. cinnamomi could be isolated from those sites in which the fungus was not recovered during the first sampling.

In conjunction with the resampling two soil sampling techniques were compared as to their suitability in detecting the occurrence of P. cinnamomi in each site. Eight soil samples were collected from three localities (Table 2.4.1) spaced at 3 m intervals, in each site. Three of these samples were prepared by bulking of cores, as described in Section 2.3.1, and five from single soil cores of approximately 100 gm each. The samples were returned to the laboratory in sealed aluminium canisters and baited with blue lupins for the presence of P. cinnamomi. Soil water potential measurements were made in the field using the filter-paper equilbration technique of Fawcett & Collis-George (1967) with five replicates for each site.

2.4.2 Results

During the first survey, carried out in 1971, P. cinnamomi had been recovered from only two of the ten sites which were resampled in November, 1972. In the resampling P. cinnamomi was again recovered from these two sites as well as from six of the other eight sites in which the fungus was not detected in the first survey (Table 2.4.1). Soil water potential readings ranged from -0.13 bars to -1.62 bars reflecting rainfall of 44.5 mm recorded for a 144 hr period two days prior to the resampling (Bureau of Meteorology, Sydney).

A comparison of soil sampling techniques indicated that the frequency of recovery of P. cinnamomi was consistently greater with bulked soil cores than with single soil cores. Thus the probability of detecting the fungus in a site are greatly increased by baiting a sub-sample of bulked soil cores compared with an equivalent number of single soil cores.

TABLE 2.4.1 Recovery of *P. cinnamomi* from ten sites sampled in 1971 and resampled in November, 1972.

Species association	Topographic location	*P. cinnamomi* recovered		*P. cinnamomi* recovered by Soil core bulking			*P. cinnamomi* recovered by Single soil cores			Water potential (bars)
		1971	1972	*A	B	C	*A	B	C	
E. maculata	midslope	-	+	+	-	-	- -	- -	-	- 1.62
E. saligna	midslope	-	-	-	-	-	- -	- -	-	- 0.38
E. maculata	midslope	-	+	+	+	+	+ +	- -	+	- 0.25
E. maculata	midslope	+	+	+	+	+	+ +	+ +	+	- 0.50
E. maculata	ridge	-	+	+	-	+	- +	- -	-	- 1.04
E. maculata	gully	-	+	+	+	+	+ +	+ -	+	- 0.13
E. maculata	midslope	+	+	+	+	+	+ +	+ +	+	- 0.17
E. maculata	midslope	-	+	-	-	+	- -	- -	-	- 0.22
E. maculata	midslope	-	-	-	-	-	- -	- -	-	- 0.22
E. gummifera	ridge	-	+	-	+	+	- -	- +	+	- 0.20

* Comparable sampling localities within site

2.5 INTENSIVE SAMPLING OF A SINGLE LOCALITY

2.5.1 Experimental

Intensive sampling to determine the distribution of *P. cinnamomi* at different topographic sites within a single locality was carried out in c. 2 ha. area located in Boyne State Forest, 22 km south of Termeil. Sampling for *P. cinnamomi* was carried out on three successive occasions, in April, 1971, May, 1972 and June, 1972, and was restricted to the northern aspect of the slope along transects running from ridge top to gully. In the first and second samplings one soil sample (c. 100 gm) from a single core was obtained at each sampling point. In the third sampling soil cores were bulked using the technique described in Section 2.3.1. The soil samples were returned to the laboratory in sealed aluminium canisters and baited with blue lupins for the presence of *P. cinnamomi*. Data on the vegetation distribution pattern within the site, collected by students of the Forestry Department, A.N.U. (Furrer, 1971), showed that the *Eucalyptus maculata* - *E. paniculata* association was dominant although *E. muellerana* was also abundant.

2.5.2 Results

The recovery of *P. cinnamomi* with bulked soil cores was greater than with single soil cores, confirming the results obtained in Section 2.4.1. Similarly *P. cinnamomi* occurred thoughout the site independent of topographic location although there was a consistent decrease in the number of single soil cores from which the fungus could be isolated with increasing distance from the gully.

TABLE 2.5.1 Proportion of samples containing *P. cinnamomi* from a site sampled on three successive occasions

Topographic location of samples	Date sampled and sampling technique used					
	April, 1971; single core		May, 1972; single core		June, 1972, bulked cores	
Upper midslope	2/8	(25%)	0/5	(0%)	4/5	(80%)
Midslope	3/8	(38%)	2/5	(40%)	4/5	(80%)
Lower midslope	3/8	(38%)	5/10	(50%)	9/10	(90%)
Gully	4/8	(50%)	4/5	(80%)	5/5	(100%)

2.6 DETERMINATION OF GROWTH RATES AND MATING TYPE OF ISOLATES OF P. CINNAMOMI

2.6.1 Experimental

The technique of Shepherd and Pratt (1974) was used to determine the radial growth rate of P. cinnamomi isolates grown on 2% oxoid corn meal agar at 25°C. Measurements were made on ninety-seven isolates obtained from sixty sites (Section 2.3.1).

The mating type of each of one hundred and thirty-eight isolates of P. cinnamomi, obtained during the field surveys (Sections 2.3.1 and 2.4.1), was determined using the technique described by Pratt et al.(1972 b). Each isolate was mated with an A1 and an A2 isolate obtained from the United States of America (Prof. G.A. Zentmyer) as well as with an A1 and an A2 isolate obtained from Australia (Dr B.H. Pratt).

2.6.2 Results

A histogram showing the frequency distribution of the radial growth rates of isolates obtained from the field surveys was plotted (Fig. 2.2). The mean radial growth rate of thirty-four A2 isolates, which were obtained from an area contiguous with the study area (Shepherd and Pratt, pers. comm.), did not differ significantly from that of the ninety-three A2 isolates retained from the field sampling. Insufficient A1 isolates were obtained from the study area for comparison with the A1 isolates examined by Shepherd et al. (1974) (Table 2.6.1).

TABLE 2.6.1 Mean radial growth rates of isolates of *P. cinnamomi* grown on 2% corn meal agar at 25°C

Source of data	No. of isolates examined	Mean radial growth rate (mm/24 hr)	Standard deviation	Skewness
A1 and A2 isolates of *P. cinnamomi* retained from field sampling	97	8.30	0.88	-2.49
A2 isolates only	93	8.43	0.58	-0.85
A1 isolates only	4	5.32	1.46	-
Shepherd and Pratt (1974) Southern N.S.W., A2 isolates only	34	8.39	1.25	-1.45
Shepherd and Pratt (Unpubl. data) Australia, A2 isolates only	332	8.15	1.43	-
Shepherd *et al.* (1974) A1 isolates	22	6.88	0.92	-

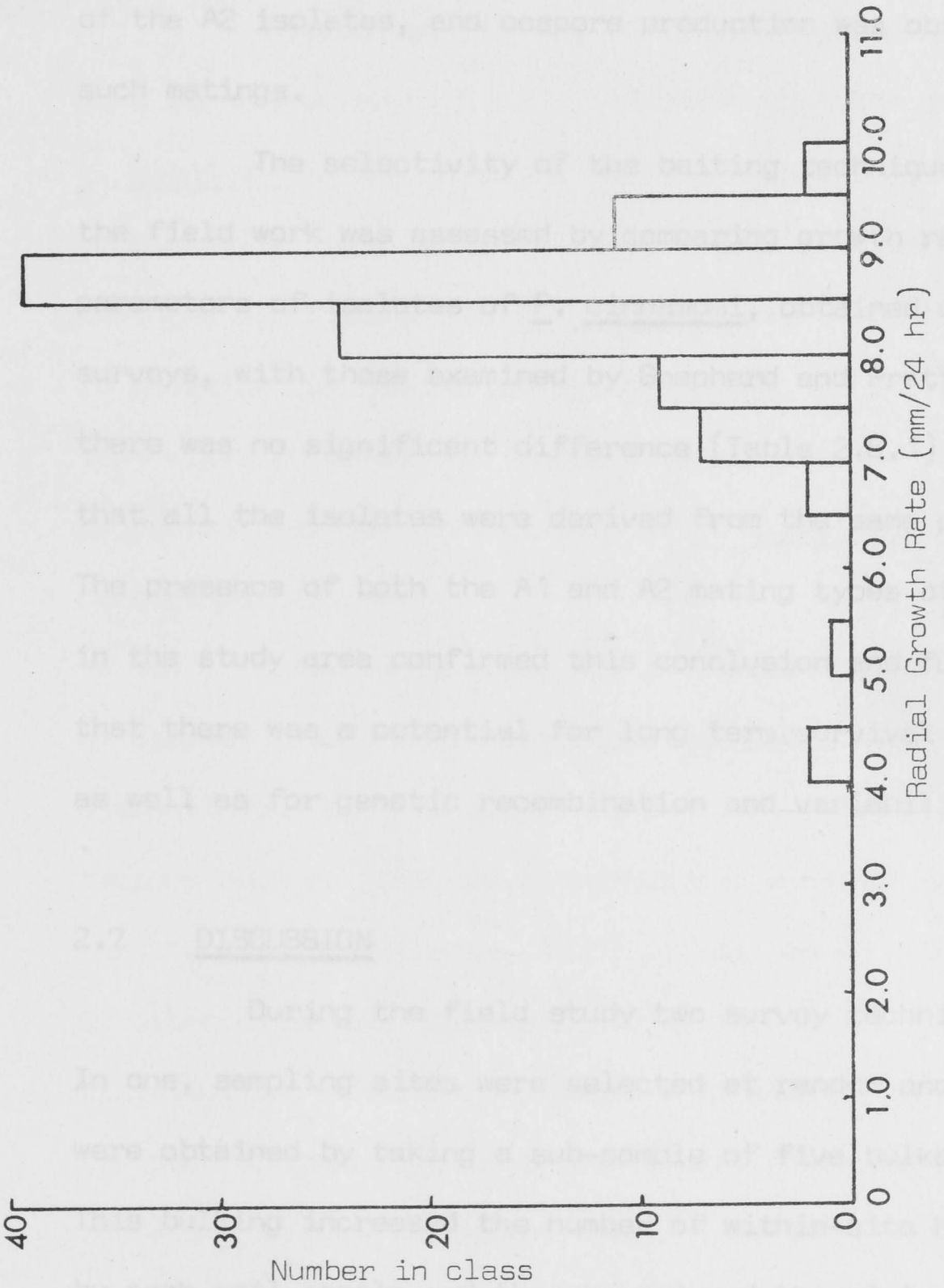


FIG 2.2 Frequency distribution of radial growth rates of 97 isolates of P. cinnamomi grown on corn meal agar at 25°C

In an examination of the mating type of one hundred and thirty-eight isolates of P. cinnamomi, five were A1 and the remaining one hundred and thirty-three were A2. The mating types were confirmed by mating the five A1 isolates with a number of the A2 isolates, and oospore production was observed in all such matings.

The selectivity of the baiting technique used during the field work was assessed by comparing growth rate distribution parameters of isolates of P. cinnamomi, obtained during the surveys, with those examined by Shepherd and Pratt (1974). As there was no significant difference (Table 2.6.1) it was concluded that all the isolates were derived from the same population. The presence of both the A1 and A2 mating types of P. cinnamomi in the study area confirmed this conclusion and further suggested that there was a potential for long term survival of the fungus as well as for genetic recombination and variability.

2.7 DISCUSSION

During the field study two survey techniques were used. In one, sampling sites were selected at random and soil samples were obtained by taking a sub-sample of five bulked soil cores. This bulking increased the number of within-site habitats covered by each soil sample and thereby reduced the minimum number of samples required for the detection of P. cinnamomi within the site.

In addition the restriction, imposed by subsequent processing facilities, on the number of sites which could be assayed in any period was eased. With the second technique sites were sampled using both bulked and single soil cores.

A comparison of the two soil sampling procedures, when used simultaneously (2.4.1), indicated that the detection of P. cinnamomi at any sampling point within a sampling site was consistently lower using the single soil core technique, suggesting that the distribution of P. cinnamomi was discontinuous in space. In addition the recovery of P. cinnamomi, in the same survey, from sites in which the fungus was not detected when first sampled suggested that the distribution of P. cinnamomi in soil was discontinuous in time.

These conclusions are in agreement with the concept of Garrett (1956) that substrates for soil fungi are essentially discontinuous both in space and in time. Other environmental factors such as moisture, temperature, aeration and the mechanical structure of soil (Raney, 1965) as well as pH and the presence of other organisms (Chapman, 1965) may also influence fungal distribution. Within any local environment there would be variable interactions of these factors, both in space and time, resulting in many different types of ephemeral microhabitats, each with its own microbial community (Park, 1968).

When the two survey techniques were used, different environmental parameters were found to be associated with the distribution of P. cinnamomi. Results with the first survey technique suggested that the distribution of the fungus was not obviously related to any of the macro-environmental parameters examined, except for an inverse correlation ($P = 0.05$) with soil moisture (2.3.2). However the second survey technique showed a consistent decrease in the detection of P. cinnamomi with increasing distance from the gully when single soil cores were used, indicating that the distribution of P. cinnamomi was apparently non-random i.e. there was an association between fungal distribution and topography with the greatest number of microhabitats favourable for P. cinnamomi occurring in the gully. A similar trend, although not as consistent, was obtained using the bulked soil core technique (2.5.1). Thus the assessment of the relative importance of the influence of each environmental factor on the distribution of P. cinnamomi varies with the sampling technique used and with the time of sampling. On this basis the environmental factors apparently associated with the distribution pattern of P. cinnamomi in the study area which would emerge from a non-random, single-core, sampling technique would show further differences from those reported in the results.

As the distribution of P. cinnamomi, as determined by the bulked soil core sampling technique, did not appear to be related to most macro-environmental factors studied it may be that, within the limitations of the isolation technique, P. cinnamomi should be recoverable in the study area from any randomly selected site of specified minimum size. This was supported by the result of a more intensive resampling of a number

of sites (2.4.1), in which the fungus was detected in sites from which it was not previously recovered in the survey reported in section 2.3.1.

During the period of the field surveys little manifest disease was evident in the sampling sites, which suggested that there was a relatively stable interaction between P. cinnamomi, host plants and the environment. This suggestion was supported by reports of manifest disease in sites, within and adjacent to the study area, which had been more recently disturbed by construction of logging tracks, roads and culverts (Pratt et al., 1973). The apparently non-random (c.f. Baker, 1971) distribution of P. cinnamomi in the soil (2.5.1) may be characteristic of stable situations. For example the uneven distribution of Fusarium oxysporum f. cubense (E.F. Smith) Wollenw. in unplowed banana plantation soils was found to be related to the uneven distribution of substrate in an undisturbed soil (Trujillo & Snyder, 1963). Consequently average percentage recovery figures of P. cinnamomi from single soil samples obtained by sampling of large stable areas would be of little value in demonstrating the spatial distribution of the fungus (c.f. Pratt et al., 1973).

The conclusions on the apparently non-random distribution of P. cinnamomi in an area in which the host-pathogen-environment interaction appeared to be relatively stable (Section 2.5.1) may not be valid for areas where this interaction is non-stable. This instability, which would be evident as manifest disease, may be associated with the environmental disturbance of a previously stable site, or with the invasion of a site by P. cinnamomi. In these situations the distribution of the organism, at any point of space or time, would be constantly

changing. Single core random sampling of such sites may yield a high rate of recovery of P. cinnamomi as conditions for inducing instability may have produced a more uniform favourable environment for the fungus and have eliminated minor microhabitat differences which previously controlled its distribution.

Non-random sampling of these non-stable sites in which disease associated with P. cinnamomi is manifest may not enable definition of the environmental factors associated with the distribution of the fungus, either in space or time. Consequently it would not be valid to speculate on the past or future distribution of the fungus from results obtained by such sampling unless the factors affecting the equilibrium were known. However several workers (Newhook & Podger, 1972) have extrapolated the results obtained by non-random sampling of non-stable areas and suggested that these observations indicate that P. cinnamomi was introduced to Australia.

The apparent trend, shown by both single and bulked soil core sampling techniques, for increased recovery of P. cinnamomi progressing from ridge top to gully (Section 2.5.1) may reflect a gradation in micro-environmental factors of, for example, increased soil moisture and nutrient status and reduced soil bulk density from ridge top to gully (McColl, 1969). The factor in the gullies most likely to influence the activity and distribution of the fungus is higher moisture levels. This suggestion is supported by evidence presented by Pratt and Heather (1973 a) who reported that P. cinnamomi was detected most commonly in moist sites. Similarly, with the results reported in Section 2.4.1, the period of high rainfall immediately prior to the resampling of sites

from which P. cinnamomi was not recovered initially may have stimulated activity of the fungus increasing the probability of recovery.

The proposal that the microhabitat frequency distribution is related indirectly to topography but dependent on moisture level contrasts the results of the broad field survey (Section 2.3.2) in which an inverse relationship was found between soil moisture and the recovery of P. cinnamomi. However, the assessment of soil moisture levels in that survey was rated subjectively on a scale of 1-3 and may have been influenced by soil texture and hence are of questionable accuracy and value. Alternatively some feature of gullies other than moisture may be the important micro-environmental factor.

The amount and type of inoculum required for disease development has not been determined, although several workers have attempted to directly relate measures of population density of P. cinnamomi in soil with disease (c.f. Marks et al., 1972; Weste et al., 1973). These measurements, using the method of Tsao (1960), were based indirectly on numbers of infective fungal propagules present in a soil sample, and no attempt was made to catergorize propagule type or the state of activity of the fungus. However, the disease potential of the organism may not depend only on the absolute number of infective propagules, but also on the spatial distribution and frequency of microhabitats which, under favourable environmental conditions, would provide separate foci for infection of plant roots (Menzies, 1970; Baker, 1971). A change in the stability of the environment resulting, for example, from road construction or drainage re-alignment, may alter the frequency of occurrence of microhabitats favourable for fungal activity, thus altering

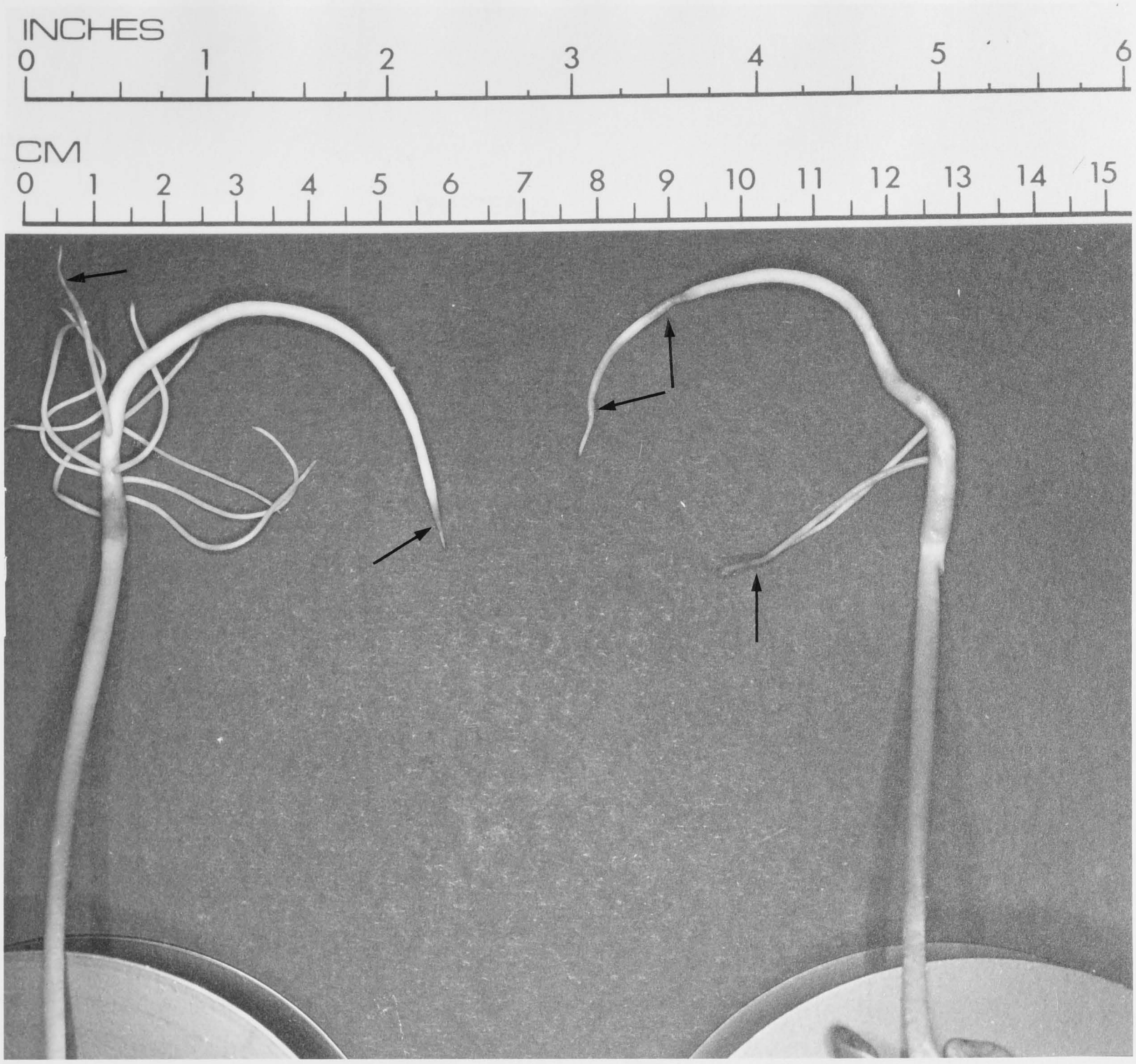
the potential for disease development by the pathogen. Attempts have been made to mathematically relate the spatial distribution of a pathogen (i.e. inoculum density) to the development of disease in a susceptible host (Baker, 1971). The use of these models would not be applicable in this instance of a stable forest situation because the basic assumption of random distribution of inoculum in the soil is not fulfilled.

Although, at the time of the surveys, the equilibrium between the host, the pathogen P. cinnamomi and environment appeared to be generally stable the present distribution of tree species may reflect instability in the past (Pratt et al., 1973). For example, the highly susceptible tree species Eucalyptus sieberi (Pratt & Heather, 1973 a) occurred in only two of the sites sampled during the initial survey (Section 2.3.1) and was limited to the ridges. Similarly only resistant and tolerant tree species, E. maculata and E. saligna (Pratt & Heather, 1973), were found in the gullies. The sharply delimited distribution of E. gummifera and E. maculata, in an area contiguous with the study area, was reported not to be associated with any marked edaphic discontinuity (McColl & Humphreys, 1967). Possibly the vegetation distribution in the area was influenced by biotic factors including P. cinnamomi. Other Phytophthora and Pythium spp. in the sampling sites (Section 2.3.2) also may have influenced the vegetation distribution pattern (c.f. Pratt & Heather, 1973 b).

Information which can be obtained from field surveys on the environmental factors influencing the activity and distribution of P. cinnamomi in soil is limited. The discontinuous distribution of P. cinnamomi in soil reflects an interaction with a non-uniform

environment on a micro-scale. Ideally then, survey techniques should be of sufficient sensitivity to detect these environmental differences as well as the state of activity of the fungus in the soil. However surveys of this type, apart from their impracticability, would modify the environments in the process of setting up survey equipment.

The difficulties created by such an approach could be reduced by associated laboratory and glasshouse experiments in which the relative significance of the influence of different environmental factors on both the fungus and the host could be assessed under controlled conditions. The difficulties inherent in either the field/laboratory or the laboratory/glasshouse approach are the extrapolation of the results obtained to the undisturbed, complex, natural environments of the forest.



0.005 M calcium

0.001 M calcium

Lesion development (↑) in roots of blue lupins grown for six days in modified Hoagland's solution at two calcium concentrations and inoculated with P. cinnamomi

PART B

CHAPTER 3

ROLE OF CALCIUM IN DISEASE RESISTANCE

LITERATURE REVIEW

3.1 ENZYME ACTIVITY IN PHYTOPHTHORA SPP.

Chemotactic responses of zoospores to root exudates have been demonstrated for a number of Phytophthora and Pythium species (Wood, 1967; Hickman, 1970). Zoospores of Phytopthora drechsleri and P. megasperma Drechs. var. sojae Hildebrand accumulated in the region between the root apex and root hair zone in safflower (Carthamus tinctorius L.) and soybean (Glycine max (L.) Merr.) seedlings (Mehrotra, 1970). With avocado (Persea americana) the greatest zoospore accumulation of P. cinnamomi occurred in the region of elongation (Zentmyer, 1961). Penetration of the tissue by these pathogens is probably by enzymatic breakdown of the pectic substances that are abundant in this zone of the plant root (Husain & Kelman, 1959).

In vitro pectic enzyme activity has been reported for a number of Phytophthora species (Wood, 1967). Polygalacturonase and a macerating factor were produced by P. palmivora (Butl.) Butl. when grown on a number of carbon and nitrogen sources (Akinrefon, 1969). An endo-polygalacturonase was obtained from culture filtrates of P. infestans (Mont.) de Bary grown on potato tuber tissue, although no macerating activity was detected (Cole, 1970). Pectinmethylesterase was also detected (Grossman, 1963; Clarke, 1966). In vitro cellulase

activity has been reported for only a few Phytophthora species and appears to be of little significance in host-cell degradation (Husain & Kelman, 1959).

In vitro pectic enzyme activity has been shown for P. cinnamomi (M. Veal, pers. comm.) and it would appear that pectin is a major component of the host tissue utilised by this fungus, at least during the penetration and immediate post-penetration phases of host infection.

3.2 STRUCTURE AND DISTRIBUTION OF PECTIC SUBSTANCES IN ROOTS

The basic structure of pectic substances is a chain of 1, 4 α linked residues of D-galacturonic acid with other sugars covalently attached (Bateman & Millar, 1966).

There are three types of pectic substances based on the degree of methylation of the carboxyl groups (Bateman & Millar, 1966):-

1. Pectic acid - high molecular weight polygalacturonic acid with no methylation.
2. Pectinic acid - methyl groups attached to less than 75% of the galacturonic acid carboxyls.
3. Pectin - methyl groups attached to greater than 75% of the galacturonic acid carboxyls.

Water solubility of the pectic substances increases with increasing methylation (Wood, 1967). Pectic acid and pectate salts formed with polyvalent cations such as Ca^{2+} and Mg^{2+} are insoluble in water and a number of mechanisms have been proposed to account for this decrease in water solubility (Wood, 1967). These include:-

1. Linkages between carboxyl groups and substances present in the cell wall, such as cellulose and protein.
2. Hydrogen bonding between chains of pectic substances.
3. Formation of ionic bridges between carboxyl groups of adjacent polygalacturonic acid chains through polyvalent cations such as Ca^{2+} and Mg^{2+}. This mechanism appears to be the most important in limiting solubility of pectic substances, although the other mechanisms may also be important (Wood, 1967).

Pectic substances form the main components of the middle lamella between cells, and in the primary cell wall they constitute a large proportion of substances present (Agrios, 1969). The amount and type of pectic substance which occurs in plant tissue depends on plant species and the type and age of the tissue (Wood, 1967).

3.3 DEGRADATION OF PECTIC SUBSTANCES BY FUNGAL ENZYMES

A number of enzymes are capable of breaking down pectic substances (Bateman & Millar, 1966):-

1. Pectinmethylesterases - highly specific enzymes which remove some or all of the esterified methyl groups of pectin or of pectinic acid chains. The length of the pectin chain is not affected although there is decreased solubility in water.
2. Polygalacturonases - enzymes which cleave the 1-4 glycosidic bonds of pectic substances through hydrolysis to release portions of the chain containing one or more residues of galacturonic acid. There are two types of polygalacturonases (a) endoenzymes which attack glycosidic bonds at random, and (b) exoenzymes which attack only the terminal 1-4 bond.

3. Transeliminases - enzymes which break the 1-4 bonds of pectic substances through a transeliminative cleavage, in which the cleavage at C-4 is accompanied by a simultaneous elimination of hydrogen at C-5. They have the same gross action on the polygalacturonic acid chain as polygalacturonases. The presence of calcium appears to stimulate the activity of transeliminases (Turner & Bateman, 1968).

Several factors may modify or inhibit the ability of these enzymes to degrade pectic substances. The pectic enzymes may be inactivated, inter alia, through the action of phenolic compounds or by indole acetic acid. Also accessibility to the pectic chain by enzymes may be affected by calcium bridges between carboxyl groups of adjacent chains or by the increased deposition of other polysaccharides and lignin in the walls of mature cells (Bateman & Millar, 1966).

3.4 EFFECT OF CALCIUM ON THE HOST-PATHOGEN INTERACTION

Resistance or susceptibility of plants to disease caused by a pathogen may be dependent on the rate of plant growth compared with the rate of host destruction by the pathogen (Albersheim, 1965). Thus the extent to which cell wall components are resistant to fungal enzyme degradation may determine the resistance of the host to a pathogen (Turner & Bateman, 1968).

Bateman (1964) reported enhanced resistance of bean hypocotyl tissue (Phasolus vulgaris L.) to Rhizoctonia solani Kühn after treatment of the plants with polyvalent cations, particularly calcium. He proposed that respiration was increased at points of infection resulting

in the accumulation of cations around lesions formed by the pathogen. This would result in the liberation and activation of pectinmethylesterase from host cell tissue, demethylating pectic substances in the cell walls. The formation of insoluble pectic salts through the ionic bonding of calcium with the demethylated pectic substances would prevent hydrolysis by the fungal polygalacturonase, limiting spread of the fungus.

Further evidence has been presented to support the hypothesis. Bateman and Lumsden (1965), working with bean hypocotyl tissue, reported that there was an increase in calcium content coupled with a progressive reduction in the amount of methylated pectic substances in the tissue over a 32 day period. They found that the tissue was resistant to Rhizoctonia solani after 3 weeks of growth. Thomas (1966) found that the water insoluble calcium content in fresh hypocotyl tissue of safflower was directly related to resistance to P. drechsleri. Similarly, Corden and Edgington (1960) showed that with increased calcium availability the resistance of tomato plants to Fusarium oxysporum f. lycopersici (Sacc.) Snyd. & Hans. was increased. The stems of calcium-deficient plants were found to contain more water-soluble pectin than normal plants (Edgington, Corden & Dimond, 1961).

The role of calcium as an inhibitor of polygalacturonase activity from F. oxyporum f. lycopersici was examined by Corden (1965). He found that the calcium content of tomato stem tissue was not correlated with disease or growth of the fungus in the host, and proposed that disease was reduced by inhibition of pectic enzyme activity. In vitro measurement, made at pH5, of the activity of polygalacturonase

on sodium polypectate containing different concentrations of calcium indicated that activity was lowest at high calcium concentrations. With Fusarium roseum (Lk.) emend. Snd. & Hans., calcium inhibited the activity of an endopolygalacturonase induced on polygalacturonic acid substrate at pH7 (Perley & Page, 1969). However, the activity of a second endopolygalacturonase, induced on pectin substrate at pH3.4, was stimulated by calcium. As the types of induced pectic enzyme activity may be determined by environmental conditions (Bateman & Millar, 1966), the working hypothesis used by Corden (1965) may have to be qualified to include a specification of the pH at which the polygalacturonase is induced in the host.

The susceptibility of tobacco to Phytophthora parasitica (Dast.) var. nicotianae (Breda de Haan) Tucker decreased as levels of calcium were reduced (Wills & Moore, 1969). The proposal that these results were related to increased membrane permeability induced by low calcium in the medium is at variance with other evidence. This suggests that increased membrane permeability could result in the increased leakage of materials from affected cells encouraging pathogen development (Lai, Weinhold & Hancock, 1968). Other mechanisms involving calcium appear to be implicated. Unbehaun and Moore (1970), working on black root rot disease of tobacco associated with Thielaviopsis basicola (Berk. & Br.) Ferraris, noted peaks in enzyme activity at pH5 and pH9. They showed that fungal pectic enzyme activity was stimulated by calcium at pH9 but not at pH5, and postulated that the calcium-stimulated enzyme was a transeliminase. Trans-eliminase stimulation by calcium has also been noted by other workers (Bateman & Millar, 1966; Wood, 1967) and the results reported by

Wills and Moore (1969) may reflect such a stimulation in P. parasitica var. nicotianae.

Changes in cell membrane permeability may take place in association with pathogenesis (Lai, Weinhold & Hancock, 1968). After inoculation of mung bean (Phaseolus aureus Roxb.) hypocotyls with Rhizoctonia solani there was an increase in cell membrane permeability ahead of necrosis. Although a factor altering permeability was isolated from infected tissue it could not be determined whether this factor arose from the host or pathogen (Lai, Weinhold & Hancock, 1968).

Studies on the role of calcium in plant membranes and in ion absorption showed that calcium deficiency may cause a breakdown in structure and increase membrane permeability (Jones & Lunt, 1967). The influence of calcium on changes in membrane permeability induced by pathogens has not been examined.

3.5 EFFECT OF CALCIUM AND OTHER NUTRIENTS ON THE INTERACTION OF P. CINNAMOMI WITH A HOST

In preliminary studies it was found that increased calcium availability reduced the amount of root-breakdown in blue lupins by P. cinnamomi (Bellany et al.1971, pers. comm.). However, the range of calcium concentrations over which significant changes in resistance would occur, as well as the mechanisms involved, was not determined.

There is little additional information available on the effects of calcium on the host-P. cinnamomi interaction, although other elements have been examined. For example, the number of pineapple

(Ananas sativus Schult.) roots infected by P. cinnamomi was reduced with increased potassium concentrations in the nutrient medium, although the rate of root-breakdown was not altered (Anderson, 1951). Similarly, the incidence of "littleleaf" disease in Pinus spp., which has been associated with nutrient deficiencies accentuated by the breakdown of roots by P. cinnamomi, was reduced following applications of N or P fertilizers (Newhook & Podger, 1972). It was proposed that this reduction resulted from enhanced mycorrhizal protection of the roots through reduced opportunity for infection by zoospores and improved conditions for host recovery (Newhook & Podger, 1972). However the hypothesis, that physiological changes may have occurred in the roots increasing resistance to colonization by the pathogen, was not considered.

There is a need to examine the extent to which increased calcium availability significantly enhances the resistance of a host to colonization by P. cinnamomi, and to determine whether resistance is due to physiological changes within the host or to a direct effect on the pathogen.

CHAPTER 4

EFFECT OF CALCIUM ON THE SUSCEPTIBILITY OF LUPIN ROOTS TO COLONIZATION BY P. CINNAMOMI

4.1 INTRODUCTION

The effect of calcium on some host-pathogen interactions was reviewed in Chapter 3. In this chapter the effect of calcium on the susceptibility of blue lupins (Lupinus angustifolius) to infection and colonization by P. cinnamomi is examined.

4.2 EXPERIMENTAL

Seeds of blue lupin were surface sterilized in 6% H_2O_2 for 3 hr, then germinated in trays of moist autoclaved vermiculite for 48 hr. The calcium concentrations were varied by adding $CaCl_2 \cdot 2H_2O$ to modified Hoagland's solution (Appendix II) except where otherwise stated.

Nutrient solutions (150 ml) were placed in a series of 160 ml plastic cups and covered with a paraffin wax film (Parafilm) to reduce evaporation. Three lupin seedlings were placed in each cup by inserting the radicles through slits in the Parafilm cover. The lupins were grown in the laboratory at 15°C-20°C for 6 days, then inoculated. The inoculum consisted of a suspension of zoospores of an A2 strain of P. cinnamomi (isolate 6105, from Eucalyptus maculata - E. botryoides association, Batemans Bay, 1971) in distilled water produced axenically using the method of Chen and Zentmyer (1970).

Lupins were removed from the nutrient solution, placed for 24 hrs in the zoospore suspension so that only the roots were exposed to the inoculum, then returned to the solutions. Control plants were exposed to distilled water only. After 72 hrs the lupins were removed and the length of the root lesions was recorded. Eleven cups, each containing three lupins, were prepared for each calcium concentration, eight of which were inoculated. Lupins in the three remaining cups were used as controls.

The relationship of lesion development to fungal penetration and colonization of roots was determined by plating consecutive sections of infected roots on agar at different times after inoculation. It was found that the zone occupied by the fungus closely approximated the area showing discolouration. Fungal lesions typically began close to the root tip and progressed evenly up the root surface, although the lesions were slightly more advanced along the cambium. As the length and thickness of lupin roots in calcium treatments was relatively uniform it was possible to use lesion length along the cambium as the unit for comparison of infected plants.

To determine whether changes in pH likely to affect infection processes occurred during the experiment, pH measurements of the nutrient solutions were made at 0 days (at the commencement of the experiment), at 6 days (immediately prior to inoculation) and at 10 days at the conclusion of the experiment (Table 4.1).

In a preliminary experiment a trend of reduced lesion development with increased calcium availability was demonstrated. In order to determine the range throughout which significant reduction occurred, an experiment was carried out in which lesion development was examined in solutions with calcium concentrations of 0.005 M, 0.004 M,

0.003 M, 0.002 M, 0.001 M and nil. Initially the cation content of uninoculated lupin roots, grown in calcium treatments 0.005 M to 0.001 M for 7 days, had been determined to examine the effect of calcium concentration of the nutrient solutions on cation uptake by the roots. Finely-ground root tissue (0.5 gm dry wt) was digested on a hot plate for 4 hr in 5 ml of 7:1 perchloric acid:sulphuric acid solution, then made up to volume in a 100 ml volumetric flask and analysed using a Varian Techtron AA-5 Atomic Absorption Spectrophotometer (Table 4.1).

4.3 STATISTICAL ANALYSIS

The mean, variance and 95% confidence limits of lesion length in each treatment was determined by use of basic statistics Fortran program A3.1, Sokal and Rohlf (1969). For each experiment, treatment variances were tested for homogeneity using Bartlett's test (Fortran program A3.8, Sokal & Rohlf, 1969).

Where variances were homogeneous Student-Newman-Keuls test was applied to determine whether differences in mean lesion length between treatments were significant (pp. 239-246, Sokal & Rohlf, 1969). Where variances were heterogeneous a non-parametric multiple comparison by simultaneous test procedures was carried out (pp. 395-397, Sokal & Rohlf, 1969).

4.4 RESULTS

TABLE 4.1 Calcium concentrations used in treatments, changes in pH of nutrient solutions and cation uptake by lupin roots

Calcium conc. in nutrient sol. (M)	pH at day			Cation content of uninoculated roots grown in nutrient solution for 7 days. Based on dry wt (ppm)					
	0	6	10	Ca	Mg	K	Mn	Zn	Fe
0.005	4.9	4.8	5.3	12365	10340	8575	390	70	370
0.004	5.1	4.7	5.7	9700	10170	8205	345	80	325
0.003	4.9	4.6	5.7	7720	9990	7820	360	75	350
0.002	5.0	4.8	5.8	5050	8950	8465	400	75	400
0.001	5.0	4.8	5.8	3015	8390	7830	415	75	365

Data for pH measurements made at three intervals during the experiment are shown in Fig. 4.1. There was a drop in the pH of the nutrient solutions prior to inoculation of the lupins. At the completion of the experiment the pH had increased in all calcium treatments. Placing uninoculated lupins into glass-distilled water for 24 hrs did not cause any subsequent change in the pH of the nutrient solutions.

Graphs relating concentrations of Ca, Mg and K in the roots to calcium concentration in solution are shown in Fig. 4.2. Correlation coefficients of 0.999 (Ca, $p < 0.01$), 0.950 (Mg, $p < 0.05$) and 0.558 (K, not significant) were obtained with nutrient calcium concentrations.

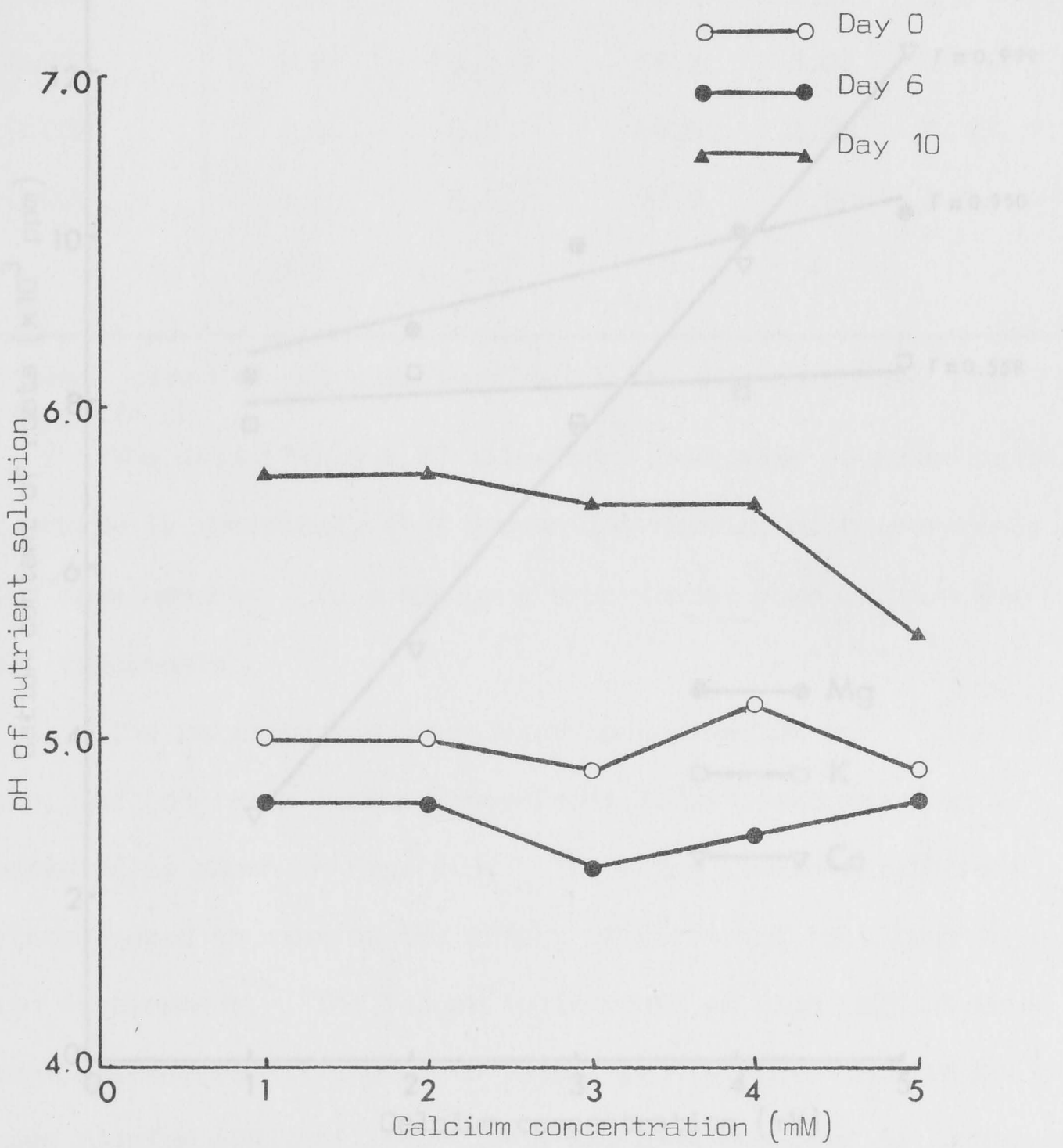


FIG 4.1 Measurement of pH of calcium nutrient solutions at day 0, day 6 and day 10

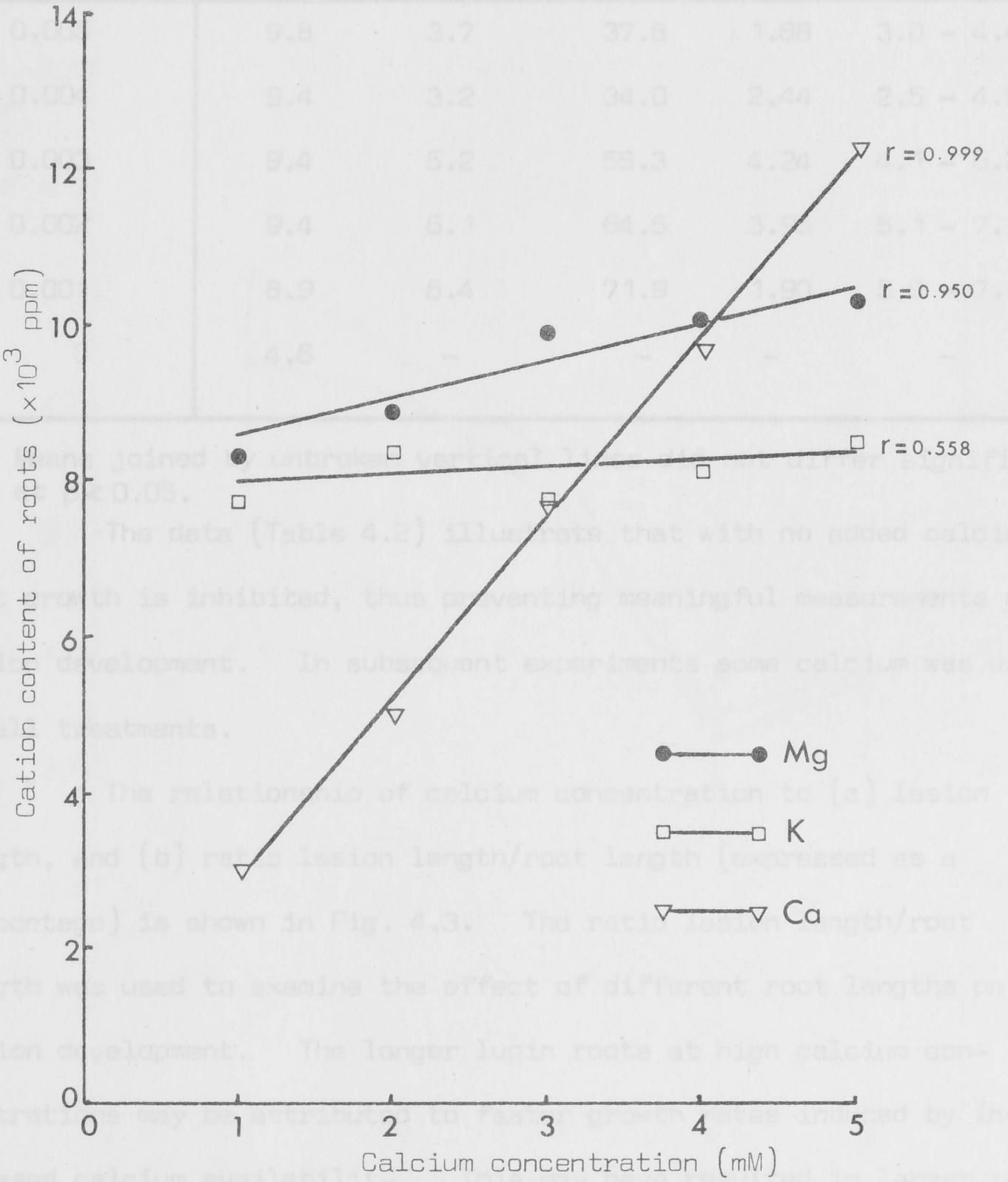


FIG 4.2 Relationship of cation content of roots to calcium concentration of nutrient solutions

TABLE 4.2 Effect of calcium on lesion development in lupins inoculated with *P. cinnamomi*

Calcium conc. in nutrient sol. (M)	A length of inoc.roots (cm)	B length of lesion (cm)	B/A x100 (%)	Variance	Confidence limits of lesion at 95% (cm) *
0.005	9.8	3.7	37.8	1.88	3.0 - 4.4
0.004	9.4	3.2	34.0	2.44	2.5 - 4.0
0.003	9.4	5.2	55.3	4.24	4.1 - 6.2
0.002	9.4	6.1	64.5	3.96	5.1 - 7.1
0.001	8.9	6.4	71.9	1.90	5.8 - 7.1
0	4.6	-	-	-	-

* Means joined by unbroken vertical lines did not differ significantly at $p < 0.05$.

The data (Table 4.2) illustrate that with no added calcium root growth is inhibited, thus preventing meaningful measurements of lesion development. In subsequent experiments some calcium was used in all treatments.

The relationship of calcium concentration to (a) lesion length, and (b) ratio lesion length/root length (expressed as a percentage) is shown in Fig. 4.3. The ratio lesion length/root length was used to examine the effect of different root lengths on lesion development. The longer lupin roots at high calcium concentrations may be attributed to faster growth rates induced by increased calcium availability. This may have resulted in larger zones of undifferentiated tissue in the root tip (Kozlowski, 1971) and increased susceptibility of the roots to infection and colonization by *P. cinnamomi*. However, the significant differences in lesion

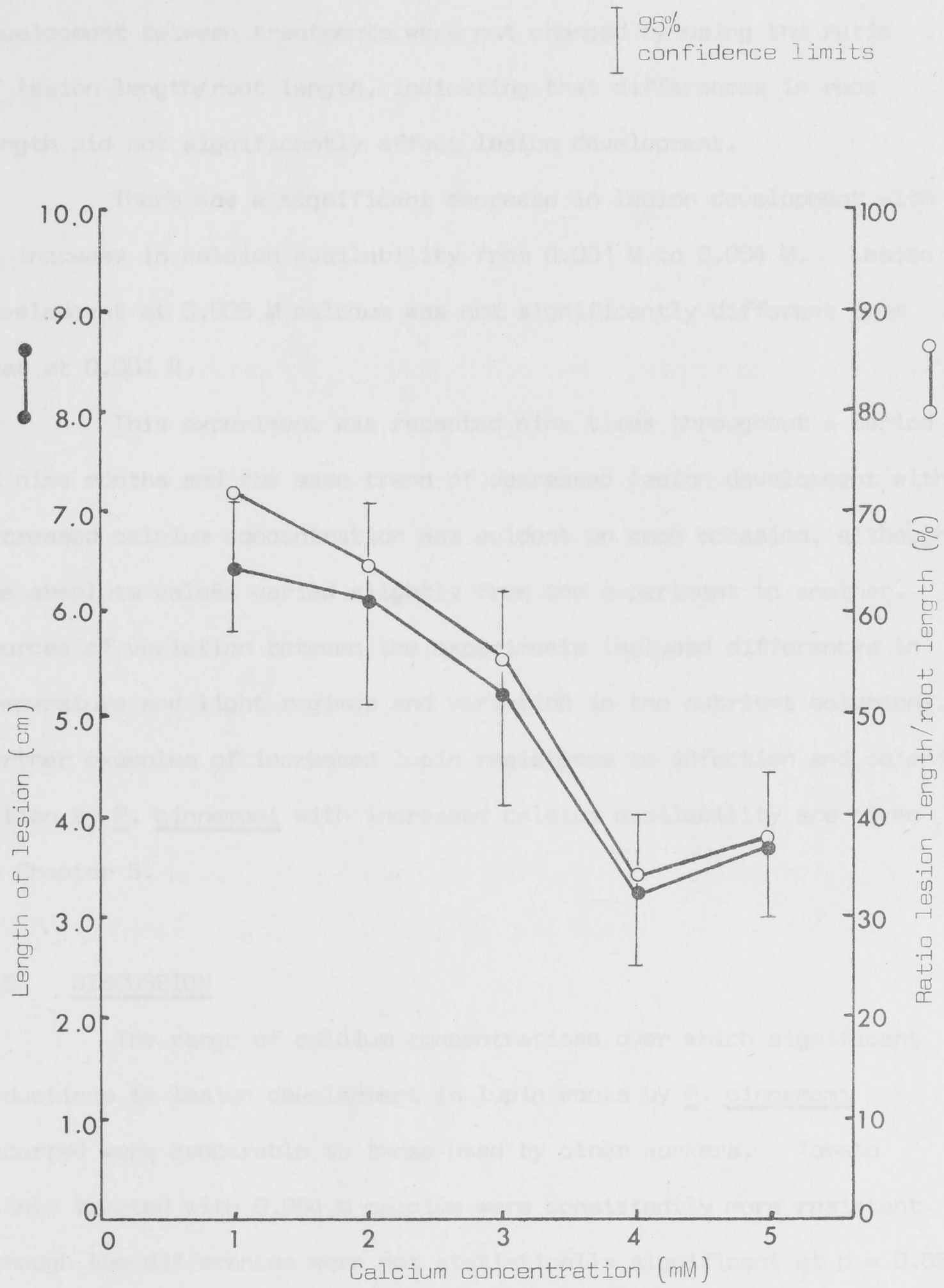


FIG 4.3 Effect of calcium on lesion development in lupins inoculated with P. cinnamomi

development between treatments were not changed by using the ratio of lesion length/root length, indicating that differences in root length did not significantly affect lesion development.

There was a significant decrease in lesion development with an increase in calcium availability from 0.001 M to 0.004 M. Lesion development at 0.005 M calcium was not significantly different from that at 0.004 M.

This experiment was repeated nine times throughout a period of nine months and the same trend of decreased lesion development with increased calcium concentration was evident on each occasion, although the absolute values varied slightly from one experiment to another. Sources of variation between the experiments included differences in temperature and light regimes and variation in the nutrient solutions. Further examples of increased lupin resistance to infection and colonization by P. cinnamomi with increased calcium availability are given in Chapter 5.

4.5 DISCUSSION

The range of calcium concentrations over which significant reductions in lesion development in lupin roots by P. cinnamomi occurred were comparable to those used by other workers. Tomato plants treated with 0.004 M calcium were consistently more resistant (though the differences were not statistically significant at $p = 0.05$) to Fusarium oxysporum f. lycopersici than plants treated with 0.001 M calcium. However significant increases in resistance were obtained in conjunction with low concentrations of boron (Edgington & Walker, 1958). Concentrations of c. 0.01 M calcium in the vascular sap of

tomato plants inhibited polygalacturonase activity from F. oxysporum f. lycopersici by 93% whereas c. 0.002 M calcium inhibited enzyme activity by 58% over a one hour period (Corden, 1965).

At 0.01 M calcium, maceration by Rhizoctonia solani of bean hypocotyl tissue was completed in 24 hours, whereas with 0.05 M calcium only slight maceration occurred (Bateman, 1964). Although less effective, magnesium supplied at the same concentrations showed a similar trend.

The linear increase in the magnesium content of the lupin roots with increasing nutrient calcium concentration (Table 4.1) suggested that magnesium may have also been a factor in the resistance mechanism. However further experiments are required to determine the mechanisms (reviewed in Chapter 3) by which calcium increases the resistance of lupins to infection and subsequent colonization by P. cinnamomi.

The increases in pH of the nutrient solutions which occurred in conjunction with lesion development may be explained on the basis of:-

(a) secondary bacterial breakdown of the plant root tissue;

(b) release of products of pectin degradation into the nutrient solution;

(c) release of fungal pectin enzymes involved in lesion development;

(d) products of fungal metabolism and possibly autolytic products.

These changes in pH may have affected the activity of P. cinnamomi in the lupin root although the extent of such an influence can be assessed only from further experiments in which pH is controlled.

4.6 CONCLUSIONS

Calcium enhanced the resistance of blue lupin roots to infection and subsequent colonization by *P. cinnamomi*. Further experiments are required to determine the mechanism of resistance.

CHAPTER 5

EXAMINATION OF RESISTANCE MECHANISMS IN THE COLONIZATION OF LUPIN ROOTS BY P. CINNAMOMI

5.1 INTRODUCTION

The mechanism, by which calcium enhanced lupin resistance to lesion development by P. cinnamomi, could not be determined from treatments in which only the calcium concentration was varied. In this chapter the effects of other variables in addition to calcium concentration were examined by:-

1. Changing the nutrient solutions every six hours during each test period so that the pH of the exogenous medium was kept relatively constant. Attempts to use buffer solutions were unsuccessful because of adverse effects on lupin growth.

2. Adjusting the initial pH of the nutrient solutions with 0.01 M NaOH.

3. Varying the magnesium concentration of the nutrient solutions to determine whether the formation of pectate bonds with the cation reduced lesion development.

4. Adding potassium to the nutrient solutions to increase membrane permeability (Rojas & Tobias, 1965).

5. Substituting a calcium chelate for calcium chloride in the nutrient solution to determine whether other forms of calcium reduced lesion development.

6. Adding an aluminium salt to the nutrient solutions to reduce both the uptake and accumulation of calcium by the plant root (Johnson & Jackson, 1964).

7. Using different isolates of P. cinnamomi as inoculum to examine variability in colonization of lupin roots.

8. Inoculating potato tubers with P. cinnamomi to determine whether changes in membrane permeability occurred in association with colonization of the tissue.

9. Adding increasing concentrations of strontium to the nutrient solutions containing a constant concentration of calcium.

5.2 EXPERIMENTAL

The techniques used in this chapter for growth and inoculation of blue lupins and assessment of lesion development are presented in Section 4.2. Unless otherwise stated lupins in eight of the eleven cups were inoculated, the remaining three cups being maintained as controls.

5.2.1 Effect of constant pH on lesion development

For each calcium concentration, shown in Table 5.1, two inoculated treatments and a control treatment were used. In treatment A the nutrient solutions were replaced at six-hour intervals following inoculation of the lupins, whereas in treatment B the nutrient solutions were not replaced. In treatment C the lupins were not inoculated. The pH of each nutrient solution was measured at the completion of the experiment (day 10). Four cups were used in treatment A, three cups in treatment B and four cups in treatment C. The effect of maintaining pH at a constant level was assessed by comparing root lesion development in treatments A, B and C (Table 5.2).

TABLE 5.1 Changes in pH of nutrient solution in association with lesion development

Calcium conc. in nutrient sol.(M)	Initial pH	Final pH		
		Treatment A	Treatment B	Treatment C
0.01	4.9	5.0	4.6	4.4
0.005	4.9	5.1	5.0	4.3
0.003	4.9	5.3	5.7	4.7
0.002	5.0	5.4	6.0	4.7
0.001	5.0	5.5	6.1	4.6
0.0003	5.0	5.7	6.4	4.8

The changes in pH which occurred in treatment B and in treatment C, which are also shown in Fig. 5.1, were comparable to those shown in Table 4.1. By changing the nutrient solutions at six-hour intervals any effects of pH increases which occurred in treatment A, on the colonization of lupin roots by P. cinnamomi would have been of short duration.

Lesion development in treatment B was consistently greater than in treatment A (Fig. 5.2), although the only significant difference occurred at 0.002 M calcium ($p = 0.01$). The difference in lesion length between the two treatments may be partly attributed to a replenishment of nutrients, especially calcium, with solution replacements in treatment A. Within each treatment there was a significant increase in resistance to lesion development with increased calcium concentrations (Table 5.2).

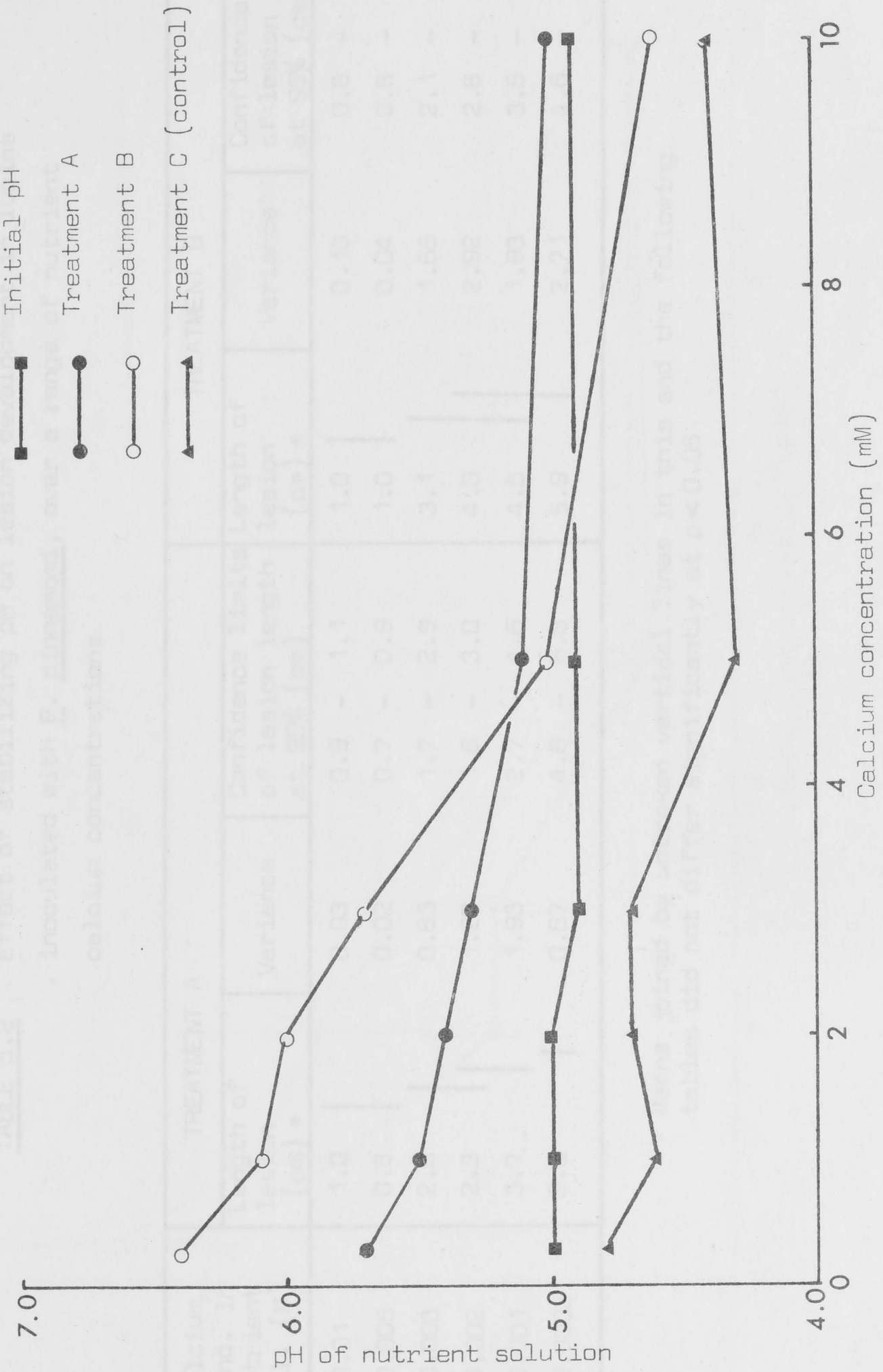


FIG 5.1 Final pH of nutrient solutions where solutions in treatment A were changed to reduce change in pH

TABLE 5.2 Effect of stabilizing pH on lesion development in lupins inoculated with P. cinnamomi, over a range of nutrient calcium concentrations

Calcium conc. in nutrient sol. (M)	TREATMENT A			TREATMENT B		
	Length of lesion (cm) *	Variance	Confidence limits of lesion length at 95% (cm)	Length of lesion (cm) *	Variance	Confidence limits of lesion length at 95% (cm)
0.01	1.0	0.03	0.9 - 1.1	1.0	0.13	0.8 - 1.3
0.005	0.8	0.02	0.7 - 0.9	1.0	0.04	0.8 - 1.1
0.003	2.3	0.83	1.7 - 2.9	3.1	1.66	2.1 - 4.1
0.002	2.3	1.27	1.6 - 3.0	4.0	2.92	2.8 - 5.3
0.001	3.7	1.93	2.7 - 4.6	4.5	1.93	3.6 - 5.4
0.0003	5.3	0.57	4.8 - 5.8	5.9	2.71	4.6 - 7.1

* Means joined by unbroken vertical lines in this and the following tables did not differ significantly at $p < 0.05$

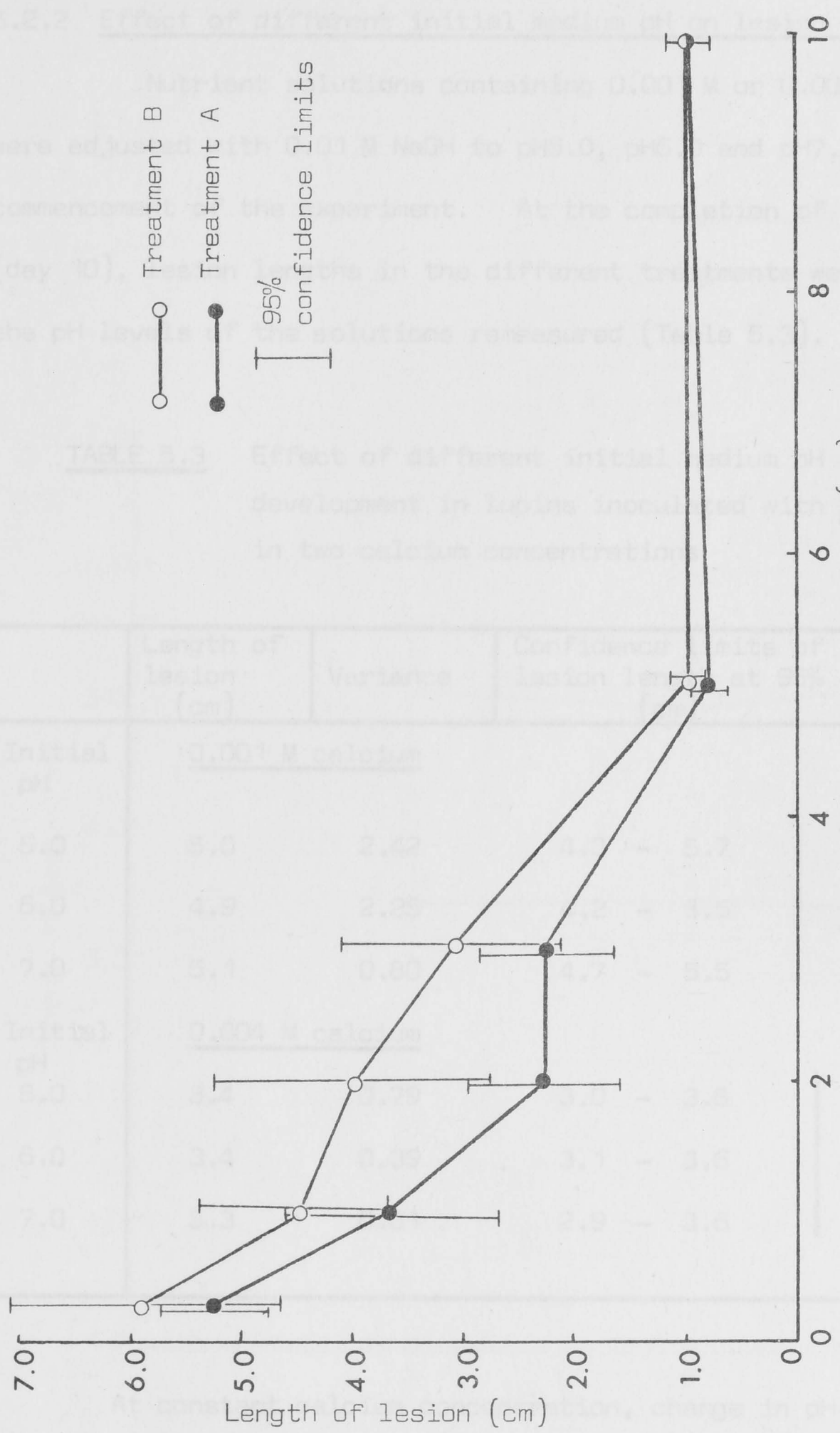


FIG 5.2 Effect of stabilizing pH on lesion development in lupins inoculated with P. cinnamomi, over a range of nutrient calcium concentrations

5.2.2 Effect of different initial medium pH on lesion development

Nutrient solutions containing 0.001 M or 0.004 M calcium were adjusted with 0.01 M NaOH to pH5.0, pH6.0 and pH7.0 at the commencement of the experiment. At the completion of the experiment (day 10), lesion lengths in the different treatments were compared and the pH levels of the solutions remeasured (Table 5.3).

TABLE 5.3 Effect of different initial medium pH on lesion development in lupins inoculated with P. cinnamomi in two calcium concentrations

	Length of lesion (cm)	Variance	Confidence limits of lesion length at 95% (cm)	Final pH
Initial pH	0.001 M calcium			
5.0	5.0	2.42	4.3 - 5.7	5.5
6.0	4.9	2.25	4.2 - 5.5	5.7
7.0	5.1	0.80	4.7 - 5.5	6.5
Initial pH	0.004 M calcium			
5.0	3.4	0.79	3.0 - 3.8	5.4
6.0	3.4	0.39	3.1 - 3.6	5.8
7.0	3.3	0.61	2.9 - 3.6	6.6

At constant calcium concentration, change in pH from pH5 to pH7 did not result in significant change in lesion length. Differences in lesion length between the two calcium concentrations were highly significant ($p < 0.01$) (Fig. 5.3).

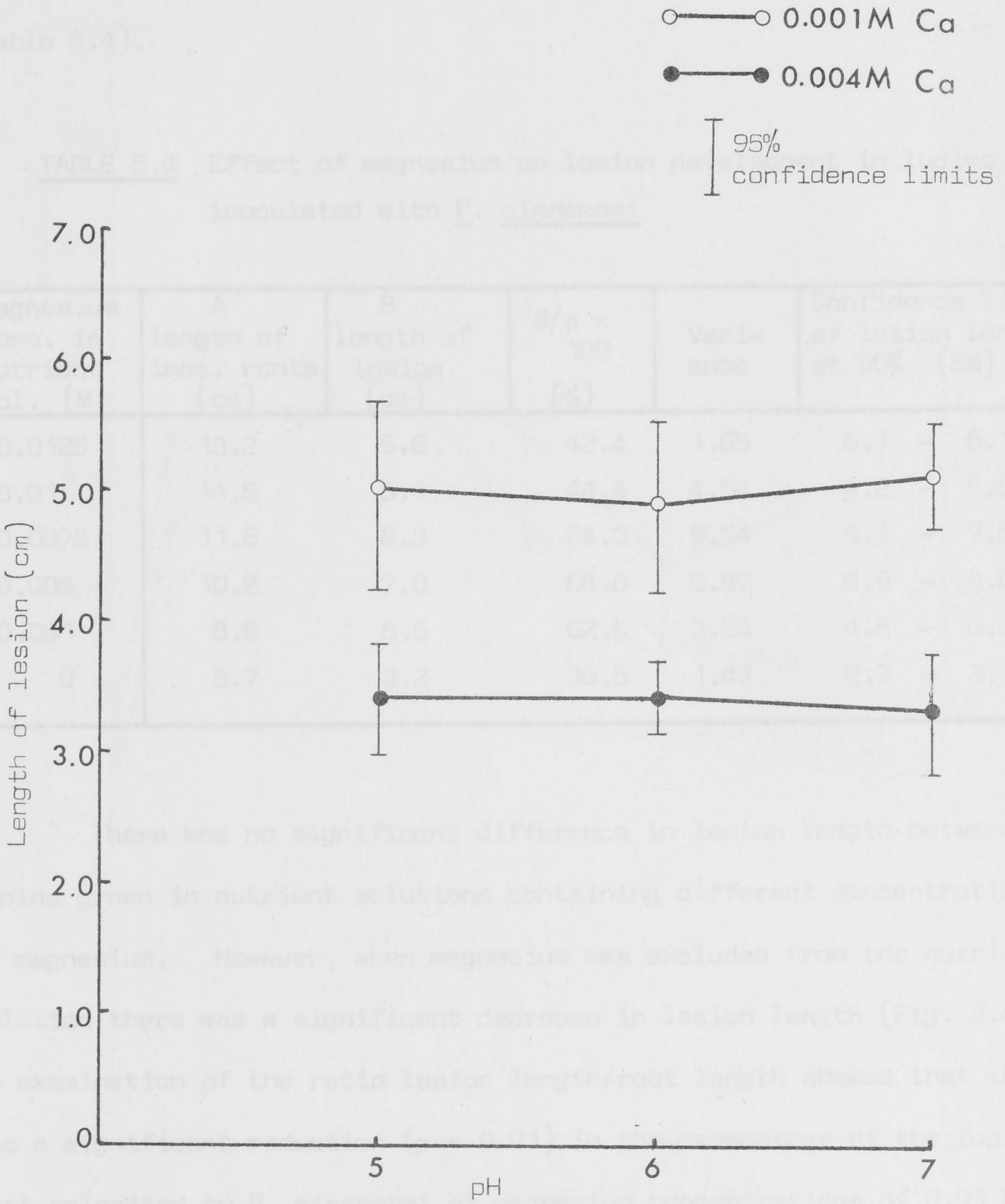


FIG 5.3 Effect of different initial medium pH on lesion development in lupins inoculated with P. cinnamomi in two calcium concentrations

5.2.3 Effect of magnesium on lesion development

The magnesium concentrations of nutrient solutions containing 0.003 M calcium were varied using $MgCl_2$ (Appendix II). Lesion lengths and root lengths in the different treatments were compared at day 10 (Table 5.4).

TABLE 5.4 Effect of magnesium on lesion development in lupins inoculated with *P. cinnamomi*

Magnesium conc. in nutrient sol. (M)	A length of inoc. roots (cm)	B length of lesion (cm)	B/a × 100 (%)	Vari-ance	Confidence limits of lesion length at 95% (cm)
0.0125	13.2	5.6	42.4	1.65	5.1 - 6.1
0.01	11.5	5.1	44.4	4.54	4.2 - 5.9
0.0075	11.6	6.3	54.3	9.54	5.1 - 7.6
0.005	10.2	7.0	68.6	6.57	5.9 - 8.0
0.001	8.8	5.5	62.5	3.24	4.8 - 6.3
0	8.7	3.2	36.8	1.47	2.7 - 3.7

There was no significant difference in lesion length between lupins grown in nutrient solutions containing different concentrations of magnesium. However, when magnesium was excluded from the nutrient solution there was a significant decrease in lesion length (Fig. 5.4). An examination of the ratio lesion length/root length showed that there was a significant reduction ($p = 0.01$) in the percentage of the lupin root colonized by *P. cinnamomi* at magnesium concentrations of 0.01 M and 0.0125 M compared with 0.005 M and 0.001 M magnesium. This may have resulted from differences in root length and subsequent differences

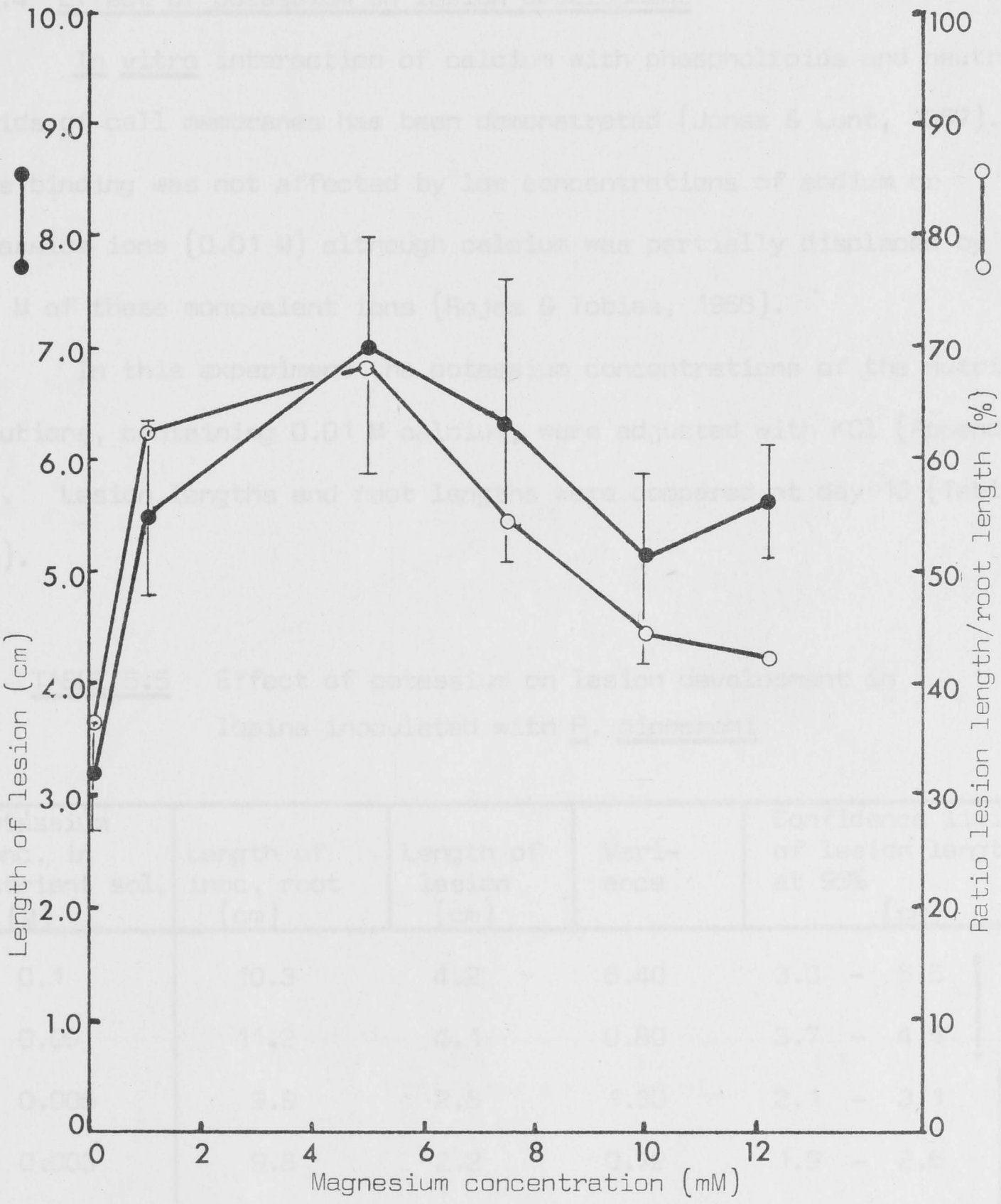


FIG 5.4 Effect of magnesium on lesion development in lupins inoculated with P. cinnamomi

in the amount of undifferentiated tissue (Kozlowski, 1971), with an enlarged zone of undifferentiated tissue in the longer roots enhancing colonization by P. cinnamomi.

5.2.4 Effect of potassium on lesion development

In vitro interaction of calcium with phospholipids and neutral lipids of cell membranes has been demonstrated (Jones & Lunt, 1967). This binding was not affected by low concentrations of sodium or potassium ions (0.01 M) although calcium was partially displaced by 0.1 M of these monovalent ions (Rojas & Tobias, 1965).

In this experiment the potassium concentrations of the nutrient solutions, containing 0.01 M calcium, were adjusted with KCl (Appendix II). Lesion lengths and root lengths were compared at day 10 (Table 5.5).

TABLE 5.5 Effect of potassium on lesion development in lupins inoculated with P. cinnamomi

Potassium conc. in nutrient sol. (M)	Length of inoc. root (cm)	Length of lesion (cm)	Vari-ance	Confidence limits of lesion length at 95% (cm)
0.1	10.3	4.2	6.40	3.0 - 5.5
0.05	11.2	4.1	0.80	3.7 - 4.5
0.006	9.9	2.6	1.30	2.1 - 3.1
0.003	9.8	2.2	0.72	1.9 - 2.6
0	9.8	2.0	0.55	1.7 - 2.3

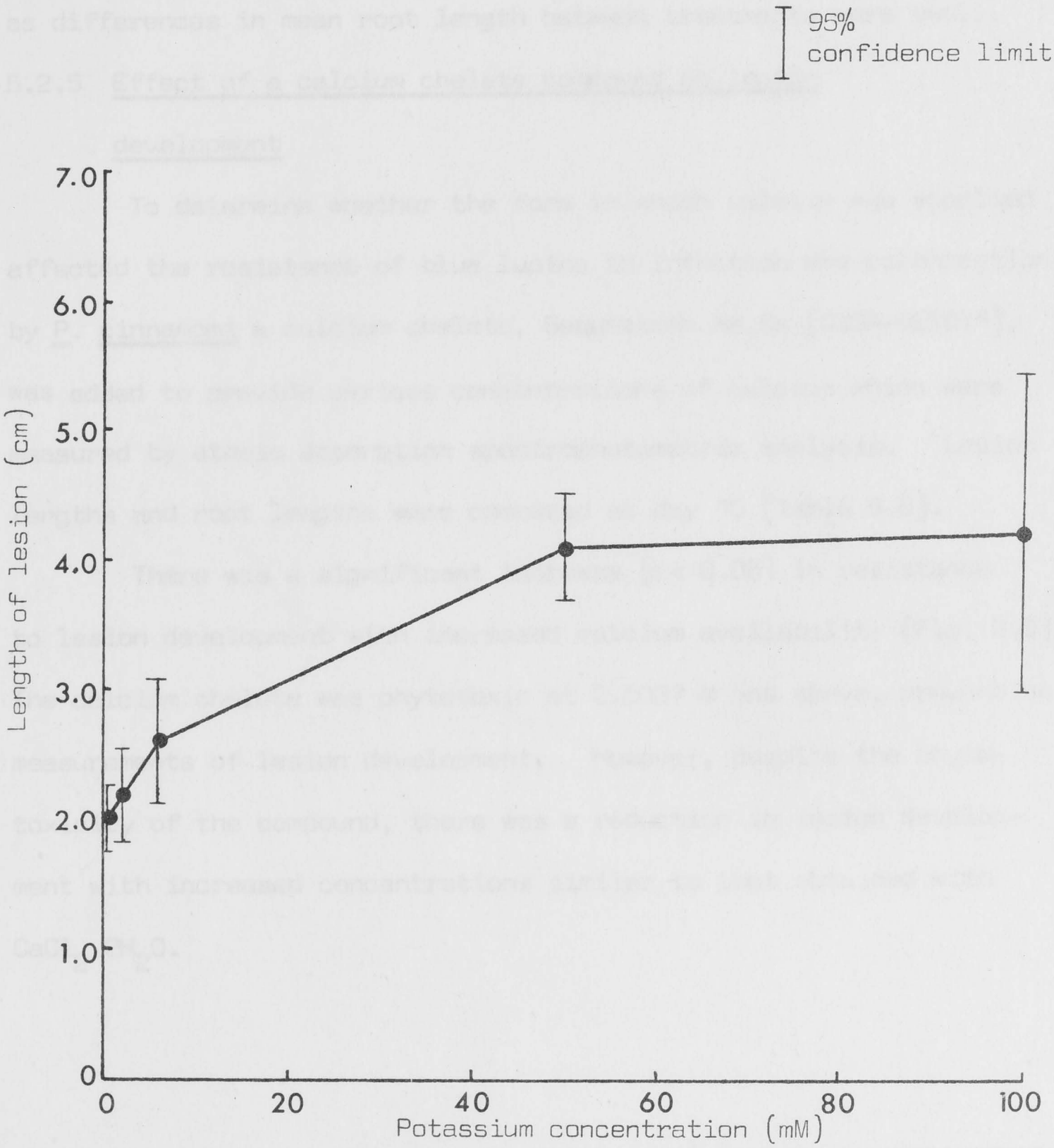


FIG 5.5 Effect of potassium on lesion development in lupins inoculated with P. cinnamomi

Lesion development at 0.1 M and 0.05 M potassium was significantly greater ($p < 0.05$) than at lower potassium concentrations (Fig. 5.5). The ratio lesion length/root length was not examined as differences in mean root length between treatments were small.

5.2.5 Effect of a calcium chelate compound on lesion development

To determine whether the form in which calcium was supplied affected the resistance of blue lupins to infection and colonization by P. cinnamomi a calcium chelate, Sequestren Na_2Ca (CIBA-GEIGY*), was added to provide various concentrations of calcium which were measured by atomic absorption spectrophotometric analysis. Lesion lengths and root lengths were compared at day 10 (Table 5.6).

There was a significant increase ($p < 0.05$) in resistance to lesion development with increased calcium availability (Fig. 5.6). The calcium chelate was phytotoxic at 0.0037 M and above, preventing measurements of lesion development. However, despite the phytotoxicity of the compound, there was a reduction in lesion development with increased concentrations similar to that obtained with $CaCl_2\ 2H_2O$.

---

* CIBA-GEIGY Australia Limited, Western Road, Kemp's Creek, N.S.W. 2171.

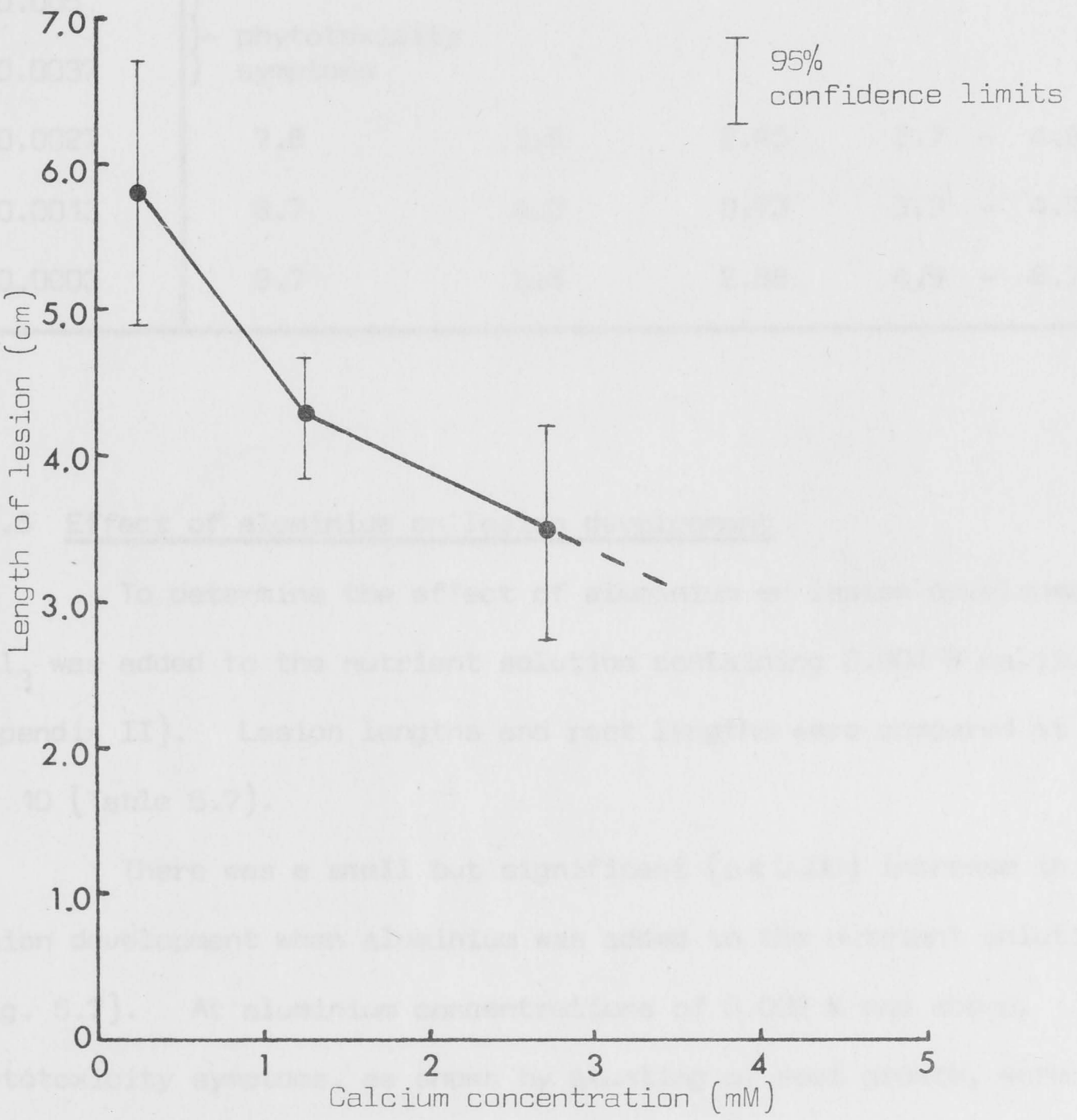


FIG 5.6 Effect of Sequestren Na_2Ca on lesion development in lupins inoculated with P. cinnamomi

TABLE 5.6 Effect of Sequestren Na_2Ca on lesion development in lupins inoculated with P. cinnamomi

Calcium conc. in nutrient sol. (M)	Length of inoc. root (cm)	Length of lesion (cm)	Vari-ance	Confidence limits of lesion length at 95% (cm)
0.005	)			
	)- phytotoxicity			
0.0037	) symptoms			
0.0027	7.8	3.5	2.45	2.7 - 4.2
0.0013	8.7	4.3	0.73	3.9 - 4.7
0.0003	8.7	5.8	2.88	4.9 - 6.7

5.2.6 Effect of aluminium on lesion development

To determine the effect of aluminium on lesion development $AlCl_3$ was added to the nutrient solution containing 0.004 M calcium (Appendix II). Lesion lengths and root lengths were compared at day 10 (Table 5.7).

There was a small but significant ($p < 0.05$) increase in lesion development when aluminium was added to the nutrient solution (Fig. 5.7). At aluminium concentrations of 0.002 M and above, phytotoxicity symptoms, as shown by stunting of root growth, were evident preventing measurements of lesion development.

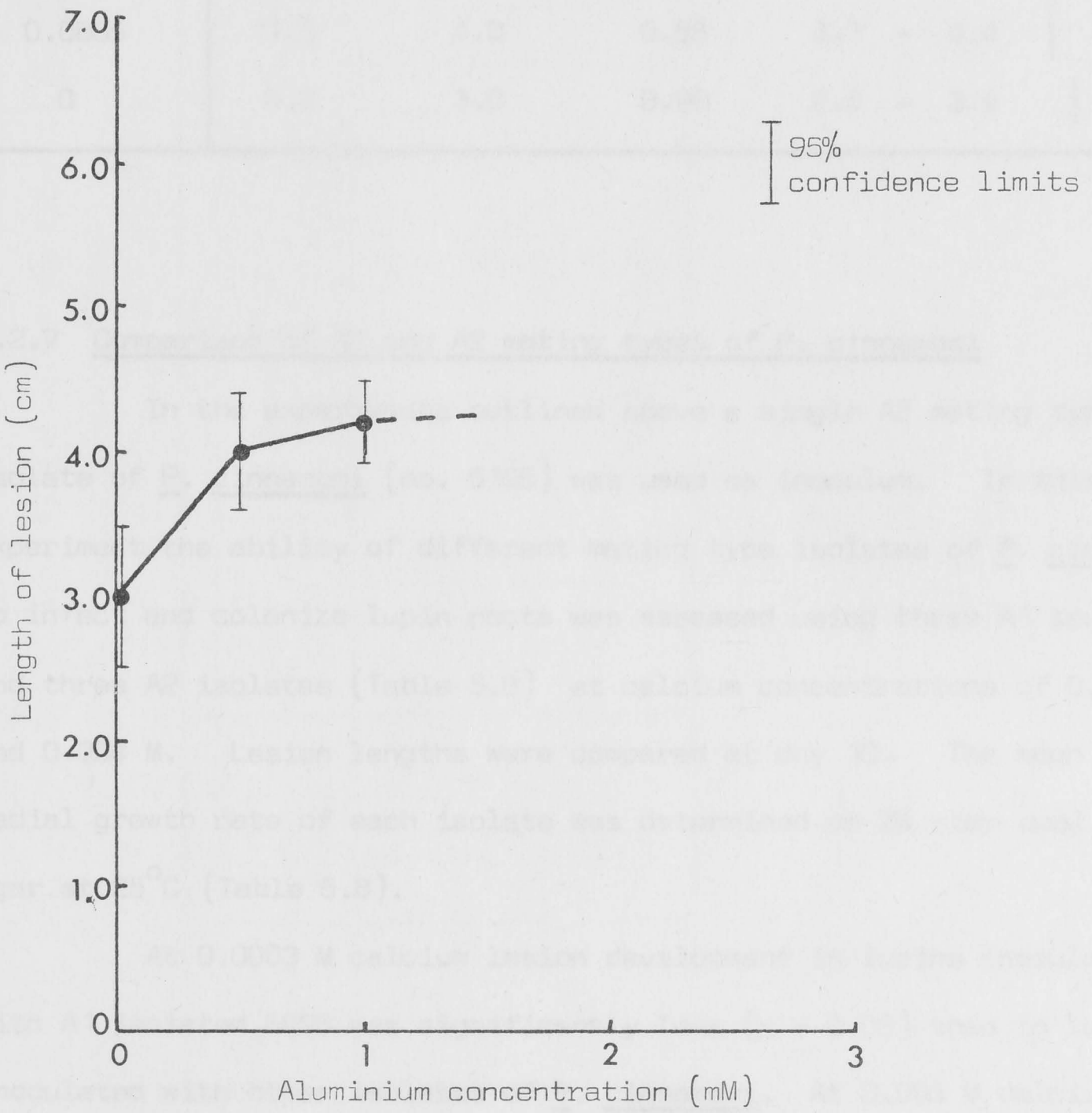


FIG 5.7 Effect of aluminium on lesion development in lupins inoculated with P. cinnamomi

TABLE 5.7 Effect of aluminium on lesion development in lupins inoculated with P. cinnamomi

Aluminium conc. in nutrient sol. (M)	Length of inoc. root (cm)	Length of lesion (cm)	Vari-ance	Confidence limits of lesion length at 95% (cm)
0.002	Phytotoxicity symptoms			
0.001	10.2	4.2	0.41	3.9 - 4.5
0.0005	11.3	4.0	0.55	3.7 - 4.4
0	8.7	3.0	0.99	2.5 - 3.4

5.2.7 Comparison of A1 and A2 mating types of P. cinnamomi

In the experiments outlined above a single A2 mating type isolate of P. cinnamomi (no. 6105) was used as inoculum. In this experiment the ability of different mating type isolates of P. cinnamomi to infect and colonize lupin roots was assessed using three A1 isolates and three A2 isolates (Table 5.8) at calcium concentrations of 0.0003 M and 0.004 M. Lesion lengths were compared at day 10. The mean radial growth rate of each isolate was determined on 2% corn meal agar at 25°C (Table 5.8).

At 0.0003 M calcium lesion development in lupins inoculated with A1 isolated 6095 was significantly less ($p = 0.05$) than in lupins inoculated with other isolates of P. cinnamomi. At 0.004 M calcium lesion development in lupins inoculated with the three A1 isolates was significantly less ($p < 0.05$) than in lupins inoculated with the three A2 isolates (Fig. 5.8). Lesion lengths with each of the A1 isolates,

TABLE 5.8 Comparison of lesion development in lupins inoculated with A1 and A2 mating types of *P. cinnamomi*, at two calcium concentrations

Isolate number	Mating type	Radial growth rate mm/day	0.0003 M calcium			0.004 M calcium		
			Length of lesion (cm)	Variance	Confidence limits of lesion length at 95% (cm)	Length of lesion (cm)	Variance	Confidence limits of lesion length at 95% (cm)
6105	A2	8.8	6.1	2.67	5.3 - 6.9	5.0	3.31	4.0 - 6.1
71-455	A2	8.0	7.3	6.25	6.0 - 8.6	4.1	5.61	2.9 - 5.3
176	A2	8.5	7.6	5.40	6.4 - 8.8	3.7	1.36	3.1 - 4.3
377	A1	7.8	6.8	6.61	5.6 - 8.1	2.2	1.46	1.6 - 2.8
375	A1	6.9	6.2	7.14	4.9 - 7.5	2.5	1.68	1.8 - 3.1
6095	A1	5.0	4.2	1.47	3.6 - 4.8	2.1	1.29	1.6 - 2.7

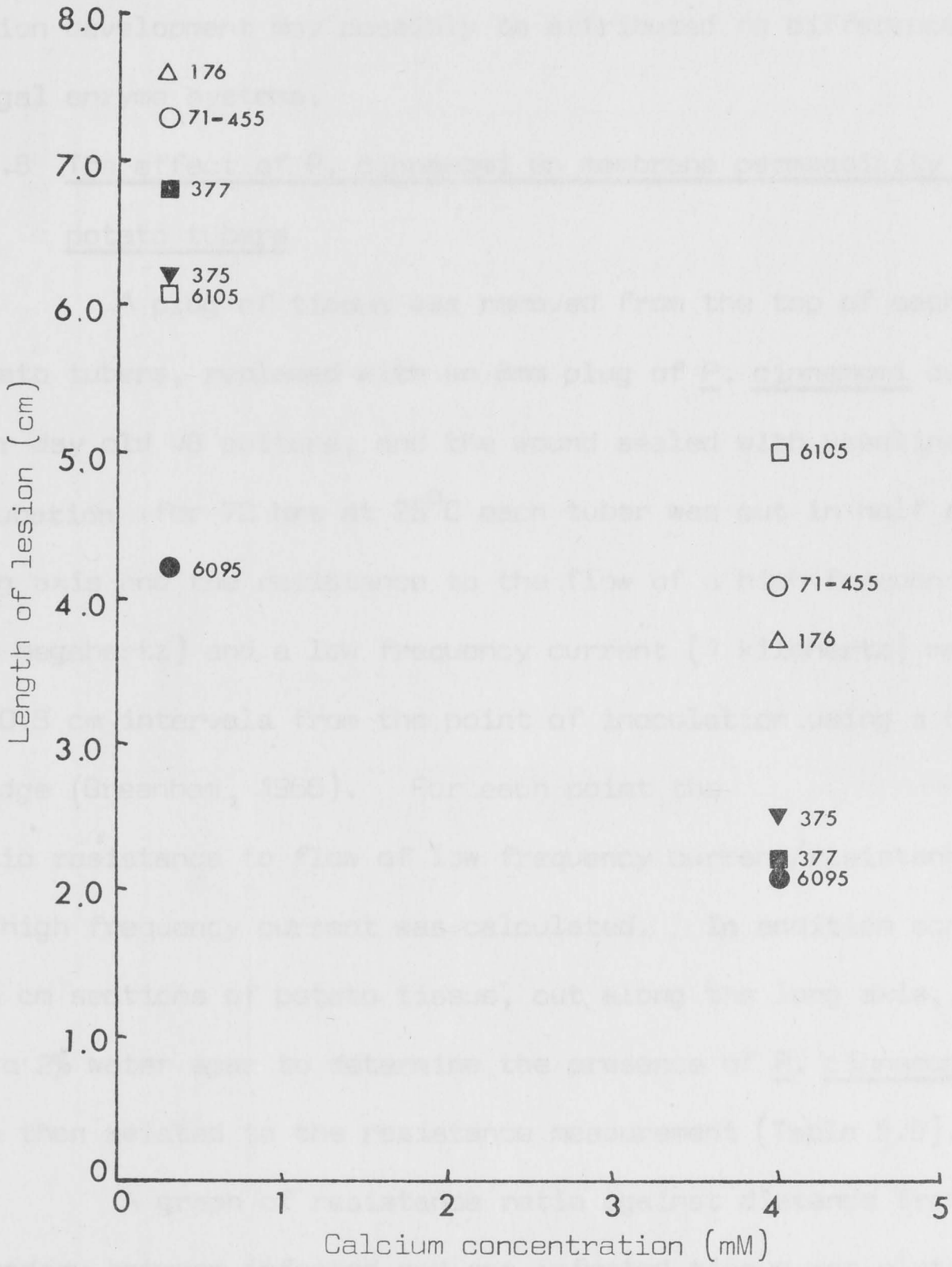


FIG 5.8 Comparison of lesion development in lupins inoculated with A1 and A2 mating types of P. cinnamomi, at two calcium concentrations

at 0.004 M calcium, were not significantly different whereas A2 isolate 176 formed a significantly shorter lesion ($p = 0.05$) than A2 isolate 6105 although it did not differ significantly from A2 isolate 71-455.

On cornmeal agar all three A1 isolates had lower mean radial growth rates than the three A2 isolates. Thus differences in lesion development may possibly be attributed to differences in fungal enzyme systems.

5.2.8 The effect of P. cinnamomi on membrane permeability in potato tubers

A plug of tissue was removed from the top of each of four potato tubers, replaced with an 8mm plug of P. cinnamomi cut from a four day old V8 culture, and the wound sealed with vaseline. After incubation for 72 hrs at 25°C each tuber was cut in half along the long axis and the resistance to the flow of a high frequency current (10 megahertz) and a low frequency current (1 kilohertz) measured at 0.5 cm intervals from the point of inoculation using a conductivity bridge (Greenham, 1966). For each point the ratio resistance to flow of low frequency current/resistance to flow of high frequency current was calculated. In addition consecutive 0.5 cm sections of potato tissue, cut along the long axis, were plated onto 2% water agar to determine the presence of P. cinnamomi which was then related to the resistance measurement (Table 5.9).

A graph of resistance ratio against distance from the boundary between infected and non-infected tissue was plotted (Fig. 5.9). Colonization of the potato tuber was associated with a significant increase in membrane permeability which occurred in conjunction with hyphal penetration of the tissue.

TABLE 5.9 Ratio of resistance to flow of low frequency current to resistance to flow of high frequency current 72 hrs after inoculation of potato tubers with P. cinnamomi

Distance from inoculum (cm)	POTATO 1		POTATO 2		POTATO 3		POTATO 4	
	Ratio	P.cinnamomi recovery by plating	Ratio	P.cinnamomi recovery by plating	Ratio	P.cinnamomi recovery by plating	Ratio	P.cinnamomi recovery by plating
0.5	1.22	+	1.12	+	1.19	+	1.13	+
1.0	1.20	+	1.17	+	1.19	+	1.10	+
1.5	1.18	+	1.23	+	1.15	+	1.13	+
2.0	2.03	+	1.40	+	1.35	+	1.20	+
2.5	8.75	+	1.64	+	1.44	+	1.20	+
3.0	10.11	+	9.19	+	1.56	+	1.41	+
3.5	10.49	-	11.16	-	3.42	+	3.17	+
4.0	13.23	-	10.68	-	8.02	+	9.14	+
4.5	13.80	-	11.39	-	9.51	+	10.96	-
5.0	11.89	-	11.69	-	10.15	-	13.05	-
5.5	10.17	-	8.47	-	12.08	-	11.15	-
6.0	12.65	-	8.52	-	13.47	-	10.00	-
6.5	11.65	-	9.05	-	12.86	-	8.40	-
7.0	a		a		11.17	-	7.06	-
7.5					9.41	-	8.08	-
8.0					9.47	-	7.39	-
8.5					8.48	-	a	
9.0					7.71	-		

\+ P. cinnamomi recovered by plating

\- P. cinnamomi not recovered by plating

a insufficient tissue

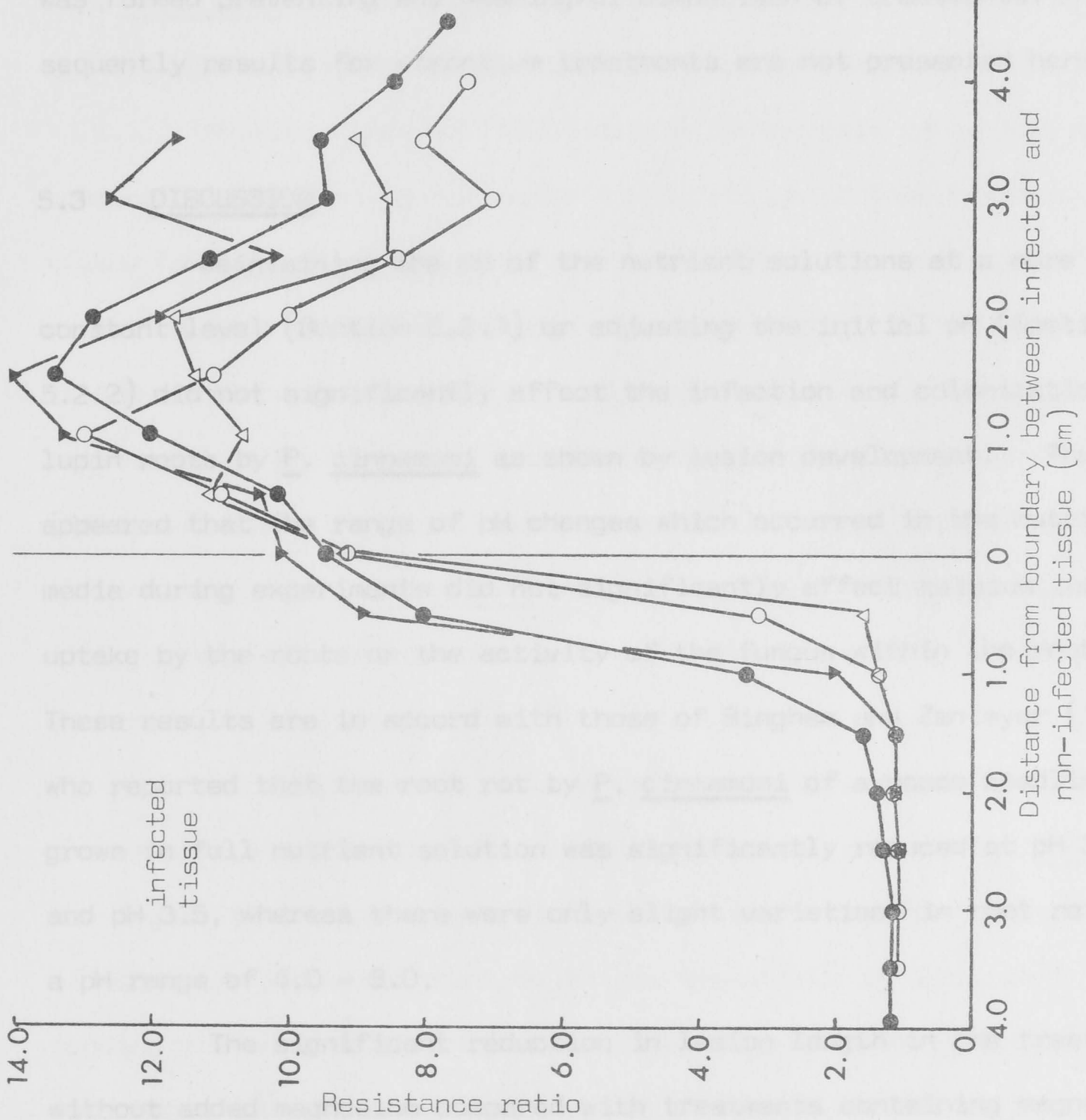


FIG 5.9 Ratio of resistance to flow of low frequency current to resistance to flow of high frequency current 72 hr after inoculation of potato tubers with P. cinnamomi

5.2.9 Effect of strontium on lesion development

Strontium has been shown to compete directly with calcium in uptake by roots (Menzel, 1954). By supplying increasing concentrations of strontium to the nutrient medium it was hoped to confirm that the resistance mechanism was associated with calcium. However, when strontium was added to the nutrient solutions a precipitate was formed preventing any meaningful comparison of treatments. Consequently results for strontium treatments are not presented herein.

5.3 DISCUSSION

Maintaining the pH of the nutrient solutions at a more constant level (Section 5.2.1) or adjusting the initial pH (Section 5.2.2) did not significantly affect the infection and colonization of lupin roots by P. cinnamomi as shown by lesion development. Thus it appeared that the range of pH changes which occurred in the nutrient media during experiments did not significantly affect calcium ion uptake by the roots or the activity of the fungus within the root. These results are in accord with those of Bingham and Zentmyer (1954) who reported that the root rot by P. cinnamomi of avocado seedlings grown in full nutrient solution was significantly reduced at pH 3.0 and pH 3.5, whereas there were only slight variations in root rot over a pH range of 4.0 - 8.0.

The significant reduction in lesion length in the treatment without added magnesium compared with treatments containing magnesium (Section 5.2.3) may possibly be attributed to the role of the cation as an enzyme activator (Jones & Lunt, 1967). Thus low concentrations

of magnesium initially may have stimulated fungal polygalacturonase activity giving an increase in lesion development. Corden, Elson and Bell (1964) reported that hydrolysis of pectin and sodium polypectate by polygalacturonase produced by Fusarium oxysporum f. lycopersici was stimulated in the presence of 0.001 M magnesium. Similarly black shank disease of tobacco caused by Phytophthora parasitica var. nicotianae was stimulated by additions of 0.007 M magnesium, although it was unaffected by 0.001 M magnesium (Wills & Moore, 1969).

However, Bateman (1964) examining the role of cation pectate bonding as a resistance mechanism for infection of bean hypocotyl tissue by Rhizoctonia solani, found that high concentrations of magnesium (i.e. >0.01 M) reduced tissue maceration by the fungus. Thus, if the ratio lesion length/root length accounted for differences between treatments in the amount of undifferentiated tissue in the root tips (Section 5.2.3), the significant reduction in the percentage of lupin root colonized by P. cinnamomi at high magnesium concentrations, compared with low magnesium concentrations, may reflect a similar resistance mechanism in lupin roots.

Although calcium and the calcium chelate compound significantly increased the resistance of the lupins to colonization by P. cinnamomi, the inability to reproduce these results with comparable concentrations of magnesium suggested that resistance mechanisms in addition to pectate bonding are important. Thatcher (1942) suggested that pectin hydrolysis only satisfied the carbon requirement of a pathogen and that other elements or energy sources from within the cell were necessary for continued fungal activity. For example, there was an increase in

membrane permeability in association with infection by Puccinia graminis Tritici of a susceptible wheat variety, whereas little change in permeability occurred when the wheat variety was inoculated with a race of P. graminis to which it was resistant. Increases in membrane permeability also occurred in potato leaf petioles inoculated with Phytophthora infestans (Thatcher, 1942).

Calcium plays an important role in membrane permeability (Jones & Lunt, 1967). For example, roots of tobacco plants treated with 0.00025 M calcium exuded four times more total sugar than roots treated with 0.0012 M calcium (Hale, Lindsey & Hameed, 1973). The close association between infection of potato tubers by P. cinnamomi and increase in membrane permeability (Section 5.2.8) suggests that changes in permeability are an important aspect of colonization of tissue by the fungus. Thus, the significant increase in lesion development which occurred when the lupins were treated with high concentrations of potassium (Section 5.2.4) suggested that the activity of P. cinnamomi was favoured by an increase in permeability. Similarly, the increase in lesion development with additions of aluminium (Section 5.2.6) may be attributed to the inhibitory effect of the cation on calcium uptake (Johnson & Jackson, 1964), increasing membrane permeability as well as reducing the number of pectate bonds in the middle lamellae.

At low nutrient calcium concentrations small increases in calcium availability may have significantly reduced membrane permeability which, in turn, may have limited the amount of root cell exudates. Magnesium, which would have a function similar to calcium in the formation

of pectate salts bonds but not in membrane permeability (Jones & Lunt, 1967), did not significantly reduce colonization, by P. cinnamomi, of the lupin roots over a wide range of concentrations. Thus it appeared that carbon pectin availability was not limiting for fungal growth, at least over the lower range of calcium concentrations. Thus, with increasing nutrient calcium concentrations, the spread of P. cinnamomi within the lupin root may be limited by its ability to alter membrane permeability and thus gain access to a secondary energy source.

A comparison of lesion development with the two different mating strains of P. cinnamomi at two calcium concentrations (Section 5.2.7) indicates a variation in ability of the fungus to induce changes in permeability in lupin roots. At the low calcium concentration there was no significant difference in lesion development whereas at 0.004 M calcium the A1 mating strain colonized significantly less root tissue than the A2 mating strain. However the effect of differences in the level of fungal polygalacturonase activity, and hence pectin utilization, cannot be excluded.

5.4 CONCLUSIONS

Changes in pectin availability through bonding of calcium with polygalactuonic acid units in the middle lamellae did not appear to be the sole mechanism by which the colonization of lupin roots by P. cinnamomi was reduced. A decrease in membrane permeability associated with increased calcium availability may also be an important mechanism in limiting colonization.

CHAPTER 6

GENERAL DISCUSSION

Field surveys on the distribution of *P. cinnamomi* in forest soils in the study area near Batemans Bay, New South Wales, indicated that the fungus was widely distributed within the area and that it could be detected in most of the randomly selected sites (Part A). This, together with the absence of widespread manifest disease of the type reported for other areas in Australia (Podger *et al.*, 1965; Podger & Ashton, 1970; Weste & Taylor, 1971) and the present overstorey vegetation distribution pattern (McColl & Humphreys, 1967) suggested that an equilibrium had been established between *P. cinnamomi*, potential hosts and environment.

In this equilibrium state the distribution of *P. cinnamomi* within a site was found to be discontinuous and non-random, the highest frequency of microhabitats favouring the fungus occurring in gullies. However it was not possible to ascertain from the field surveys which micro-environmental factors were associated with activity of *P. cinnamomi* in soil and it was suggested that this information could be obtained from further field and/or glasshouse/laboratory studies using micro-survey techniques to monitor these factors as well as the state of activity of the fungus.

The absence of widespread manifest disease may be attributed to various factors, some of which are:-

1. absence of susceptible tree species in sites with a high frequency of microhabitats favourable for activity and survival of *P. cinnamomi*;

2. occurrence of susceptible tree species only in sites with a low frequency of microhabitats favourable for activity and survival of P. cinnamomi;

3. absence of environmental stress on the host. This stress may take the form of, for example, droughting or nutrient deficiency;

4. absence of a pathogenic strain of P. cinnamomi.

The amount of inoculum required for the establishment of manifest disease has not been determined and consequently it is not possible to ascribe the absence of manifest disease to differences in the spatial distribution of P. cinnamomi alone. Similarly the significance of environmental factors on the development of manifest disease symptoms is not known.

Pratt and Heather (1973 a) reported that manifest disease associated with P. cinnamomi was most severe in areas with a climatic pattern of predominant winter rainfall followed by summer drought. Rainfall records for Batemans Bay indicate a relatively even distribution of rainfall throughout the year although a drier period occurred in winter (Anon, 1966). In addition available soil moisture levels dropped below permanent wilting point (-15 bars) for only short intervals during the year (McColl, 1969). This suggested that the tree may be under water stress for periods of insufficient duration for the development of disease symptoms.

Extensive manifest disease associated with P. cinnamomi of Eucalyptus spp. has been reported for areas with soils of low cation exchange capacities (Pratt & Heather, 1971, pers. comm.; Podger, 1972).

Similarly the incidence of littleleaf disease associated with P. cinnamomi in several Pinus spp. was reduced with applications of inorganic nitrogen fertilizers (Campbell & Copeland, 1954). This reduction in disease was attributed to reduced opportunities for infection of roots by the fungus as well as enhanced host vigour (Newhook & Podger, 1972).

In laboratory studies additions of nutrient calcium from 0.0003 M to 0.01 M significantly reduced the colonization of lupin roots by P. cinnamomi and this was attributed to physiological changes in the root (Part B). The gradation in cation exchange capacity of forest soil in the Batemans Bay area, ranging from 0.0005 M available calcium on the ridges to 0.015 M available calcium in the gully (McColl & Humphreys, 1967), suggests that the availability of calcium in the soils of the study area may have also been a factor in reducing the incidence of manifest disease in the area. To substantiate this suggestion further information will have to be obtained on the ability of different Eucalyptus spp. to utilize available calcium, and on the effects of increased calcium availability on the colonization of the Eucalyptus roots by P. cinnamomi.

Field pathogenicity trials with isolates of P. cinnamomi obtained from the study area were not carried out because of the inability to delineate the factors which cause plant mortality. However examination of the growth characteristics of isolates of P. cinnamomi from the study area showed that the range of growth rates of these isolates fell within the range spanned by isolates obtained from other regions of Australia. In addition, localised disease associated with P. cinnamomi in recently disturbed sites contiguous with the study area has been reported

(Pratt et al., 1973). Hence there was no reason to believe that isolates of P. cinnamomi from the study area differed significantly in pathogenicity from isolates obtained elsewhere in Australia.

Further interpretation of the results obtained from the field surveys was restricted by limitations inherent in the survey techniques. These techniques did not permit accurate determination of the environmental factors associated with the activity of P. cinnamomi in soil. These limitations would apply equally to field surveys which have been conducted elsewhere in Australia and hence it would not be valid to extrapolate in space and time from the determined distribution of P. cinnamomi in soil. There are difficulties also in extrapolating from results obtained from laboratory/glasshouse studies to the field situation because of the complex interactions which may occur between environmental factors in the field. For example, conditions of temperature, moisture, nutrition or pH which may be optimum for growth or reproduction of P. cinnamomi in vitro may not reflect the environmental conditions for optimum activity in vivo. Similarly increased nutrient calcium concentrations were shown to significantly reduce the colonization of lupin roots by P. cinnamomi. However extrapolation of these results to a field situation is hampered by several factors including the lack of information on the amount and spatial distribution of inoculum, the environmental conditions necessary for tree mortality, and the absence of knowledge on the effect of calcium levels on the behaviour of roots of Eucalyptus spp.

Two mechanisms proposed to account for the reduction in the colonization of lupin roots by P. cinnamomi with increasing calcium

availability from 0.0003 M to 0.01 M were:-

1. bonding of calcium with polygalacturonic acid units in the middle lamellae, reducing the amount of substrate available to the fungus as well as presenting a potential mechanical obstacle to further penetration by hyphae;
2. a reduction in membrane permeability causing a reduction in the amount of root cell exudates and reducing the amount of substrate available to the fungus.

The mechanisms could be further investigated by:-

1. determination of the water soluble pectin to water insoluble pectate ratio over a range of nutrient calcium concentrations in order to relate the amount of utilizable substrate directly to the rate of colonization of the tissue by the fungus;
2. determination of variations in membrane permeability at different nutrient calcium concentrations by electric conductivity measurements or by determination of the osmotic potential across the membrane.

The chemotactic attraction of zoospores of P. cinnamomi to the zone of elongation and differentiation of the host root suggests that variations in the rate of differentiation of cells in this zone may affect the amount of substrate initially available to the fungus, and hence the energy available for further colonization of the root. The effect of calcium on the rate of differentiation of the cells in the zone of elongation of the root tip should be examined in order to determine whether differences in the degree of cell differentiation affect the establishment of infection.

An additional mechanism of direct inhibition of P. cinnamomi by calcium would have to be examined further in an experiment in which the calcium concentration of the nutrient solutions is varied after inoculation of the lupins.

In order to determine whether increased calcium availability will enhance the resistance of roots of Eucalyptus species to colonization by P. cinnamomi a similar experimental approach to that used with lupins would be required initially. It would be necessary to examine the resistance mechanisms, associated with increased calcium availability, on Eucalyptus seedlings grown in nutrient culture. This would ensure that there is no inhibition of calcium uptake by competition with other cations such as aluminium, and that inoculation of the roots takes place. Assessment of the effectiveness of calcium as determined by the extent of lesion development would be easier to determine in this system. Should increased calcium availability significantly reduce colonization of the Eucalyptus roots by P. cinnamomi, it would be practicable to examine the effect of calcium on the development of disease symptoms, associated with P. cinnamomi, in Eucalyptus seedlings grown in a particulate system under controlled conditions. The initial use of vermiculite in such a system would ensure that the distribution of inoculum is discontinuous which may reduce the proportion of roots exposed to infection. The subsequent use of soil may introduce further variables including reduced availability of calcium through adsorption of the cation to soil particles as well as reduced calcium uptake by competition with other cations such as aluminium. In a field situation an examination of the

effectiveness of calcium applications on the resistance of Eucalyptus spp. to disease associated with P. cinnamomi may be further complicated by increased competition between plant species with increased calcium availability. In addition the form in which the calcium is applied may also be very significant in the results achieved.

The proposed approach to examine the effect of calcium on the colonization of roots of Eucalyptus spp. by P. cinnamomi, and to assess the effectiveness of the cation in reducing disease associated with the fungus in the field, underlines some of the complex interactions which may occur between environmental factors. Thus inability to show a reduction in manifest disease associated with P. cinnamomi following application of fertilizers must be accompanied by an examination of the changes which may occur in the host, fungal population and environmental factors following such applications (c.f. Newhook & Podger, 1972; Marks et al., 1973).

REFERENCES

Adebayo, A.A., & Harris, R.F. (1971). Fungal growth responses to osmotic as compared to matric water potential. Soil Science Society of America, Proceedings. 35: 465-469.

Agrios, G.N. (1969). Plant Pathology. Academic Press, New York, 629p.

Akinrefon, D.A. (1969). Factors affecting the production of extra-cellular pectolytic enzymes by Phytophthora palmivora (Butl.) Butl. Annals of Botany. 33(131): 439-450.

Albersheim, P. (1965). The substructure and function of the cell wall. pp.151-186. In PLANT BIOCHEMISTRY, Eds. J. Bonner & J.E. Varner. Academic Press, New York.

Anderson, E.J. (1951). Effect of nutrient salt concentrations on infection of pineapple roots by Phytophthora cinnamomi. Phytopathology. 41: 2 (Abstract).

Anonymous (1966). Rainfall Statistics Australia. Long period averages (all years) standard period normals of rainfall and raindays (1931-1960). Bureau of Meteorology, Melbourne.

Anonymous (1969). Climatic Averages Australia. Temperature, relative humidity, rainfall. Bureau of Meteorology, Melbourne.

Baker, R. (1970). Use of population studies in research on plant pathogens in soil. In ROOT DISEASES AND SOIL-BORNE PATHOGENS. Eds. T.A. Toussoun, R.V. Bega & P.E.Nelson. Berkeley, University of California Press. pp.11-15.

Baker, R. (1971). Analyses involving inoculum density of soil-borne plant pathogens in epidemiology. Phytopathology. 61: 1280-1292.

Bateman, D.F. (1964). An induced mechanism of tissue resistance to polygalacturonase in Rhizoctonia-infected hypocotyls of bean. Phytopathology. 54: 438-445.

Bateman, D.F., & Lumsden, R.D. (1965). Relation of calcium content and nature of the pectic substances in bean hypocotyls of different ages to susceptibility to an isolate of Rhizoctonia solani. Phytopathology. 55: 734-738.

Bateman, D.F., & Millar, R.L. (1966). Pectic enzymes in tissue degradation. Annual Review of Phytopathology. 4: 119-146.

Bellany, G., Heather, W.A., & Pratt, B.H. (1971) Personal communication. The effect of calcium chloride treatment in reducing the rate of spread of Phytophthora cinnamomi within roots of Lupinus angustifolius. Working Papers, Australian Plant Pathology Conference, Hobart. 3e 13-15.

Bingham, F.T., & Zentmyer, G.A. (1954). Relation of hydrogen-ion concentration of nutrient solution to Phytophthora root rot of avocado seedlings. Phytopathology. 44: 611-614.

Campbell, W.A., & Copeland, O.L. (1954). Littleleaf disease of shortleaf and loblolly pines. United States Department of Agriculture Circular No. 940.

Chang, S.T., Shepherd, C.J., & Pratt, B.H. (1974). Control of sexuality in the genus Phytophthora with particular reference to P. cinnamomi. Australian Journal of Botany. 22: (in press).

Chapman, H.D. (1965). Chemical factors of the soil as they affect microorganisms. In ECOLOGY OF SOIL-BORNE PLANT PATHOGENS. Eds. K.F. Baker & W.C. Snyder. Berkeley, University of California Press. pp.120-141.

Chee, K.H., & Newhook, F.J. (1965). Improved methods for use in studies on Phytophthora cinnamomi Rands and other Phytophthora species. New Zealand Journal of Agricultural Research. 8: 88-95.

Chen, D.W., & Zentmyer, G.A. (1970). Production of sporangia by Phytophthora cinnamomi in axenic culture. Mycologia. 62: 397-402.

Clarke, D.D. (1966). Production of pectic enzymes by Phytophthora infestans. Nature. 211: 649.

Cole, A.L.J. (1970). Pectic enzyme activity from Phytophthora infestans. Phytochemistry. 9: 337-340.

Corden, M.E. (1965). Influence of calcium nutrition on Fusarium wilt of tomato and polygalacturonase activity. Phytopathology. 55: 222-224.

Corden, M.E., & Edgington, L.V. (1960). A calcium requirement for growth-regulator-induced resistance to Fusarium wilt of tomato. Phytopathology. 50: 625-626.

Corden, M.E., Elson, J.E., & Bell, D.L. (1964). Inhibition of polygalacturonase by cations. Phytopathology. 54: 1432 (Abstract).

Crandall, B.S., & Gravatt, G.F. (1967). The distribution of Phytophthora cinnamomi. Ceiba. 13: 43-55, 57-70.

Eckert, J.W., & Tsao, P.H. (1962). A selective antibiotic medium for isolation of Phytophthora and Pythium from plant roots. Phytopathology. 52: 771-777.

Edgington, L.V., Corden, M.E., & Dimond, A.E. (1961). The role of pectic substances in chemically induced resistance to Fusarium wilt of tomato. Phytopathology. 51: 179-182.

Edgington, L.V., & Walker, J.C. (1958). Influence of calcium and boron nutrition on development of Fusarium wilt of tomato. Phytopathology. 48: 324-326.

Fawcett, R.G., & Collis-George, N. (1967). A filter-paper method for obtaining the moisture characteristic of soil. Australian Journal of Experimental Agriculture and Animal Husbandry. 7: 162-167.

Fraser, L.R. (1956). Phytophthora cinnamomi attacking native plants. Australian Plant Disease Recorder. 8: 12.

Furrer, B. (1971). Management and silviculture in the spotted gum forests on the south coast of New South Wales. M.Sc. Thesis. Australian National University, Canberra. 119p.

Gallegly, M.E. (1970). Genetics of Phytophthora. Phytopathology. 54: 1146-1151.

Garrett, S.D. (1956). Biology of Root-infecting Fungi. Cambridge University Press, London. 293p.

*Griffin, D.M. (1972). Ecology of Soil Fungi. Chapman and Hall Ltd, London. 193p.

Grossman, F. (1963). Über ein pektolytisches Enzym von Phytophthora infestans de By. Die Naturwissenschaften. 50: 721.

Hale, M.G., Lindsey, D.L., & Hameed, K.M. (1973). Gnotobiotic culture of plants and related research. The Botanical Review. 39: 261-273.

Harley, J.L. (1972). Fungi in ecosystems. Journal of Animal Ecology. 41: 1-16.

Hendrix, F.F., & Kuhlman, E.G. (1965 a). Existence of Phytophthora cinnamomi as chlamydospores in soil. Phytopathology. 55: 499 (Abstract).

*Greenham, C.G. (1966). Bruise and pressure injury in apple fruits. Journal of Experimental Botany. 17(51) : 404-409.

Hendrix, F.F., & Kuhlman, E.G. (1965 b). Factors affecting direct recovery of Phytophthora cinnamomi from soil. Phytopathology. 55: 1183-1187.

Hickman, C.J. (1970). Biology of Phytophthora zoospores. Phytopathology. 60: 1128-1136.

Husain, A., & Kelman, A. (1959). Tissue is disintegrated. In PLANT PATHOLOGY Vol. I. Eds. J.G. Horsfall & A.E. Dimond. Academic Press, New York. pp.143-188.

Jehne, W. (1971). Soil conditions and the occurrence of Phytophthora cinnamomi in relation to deaths in young plantations of radiata pine near Jervis Bay. Australian Forest Research. 5(3): 39-46.

Johnson, R.E., & Jackson, W.A. (1964). Calcium uptake and transport by wheat seedlings as affected by aluminium. Soil Science Society of America, Proceedings. 28: 381-386.

Jones, R.G.W., & Lunt, O.R. (1967). The function of calcium in plants. The Botanical Review. 33(4): 407-426.

Kozlowski, T.T. (1971). Growth and Development of Trees. Vol. II. Cambial growth, root growth and reproductive growth. Academic Press, New York. 514p.

Kuhlman, G.E. (1964). Survival and pathogenicity of Phytophthora cinnamomi in several western Oregon soils. Forest Science. 10: 151-158.

Lai, M.T., Weinhold, A.R., & Hancock, J.G. (1968). Permeability changes in Phaseolus aureus associated with infection by Rhizoctonia solani. Phytopathology. 58: 240-245.

McColl, J.G. (1965). An ecological study of the distribution of Eucalyptus gummifera and Eucalyptus maculata. M.Sc. Thesis. Australian National University, Canberra. 162p.

McColl, J.G. (1969). Soil-plant relationships in a Eucalyptus forest on the south coast of New South Wales. Ecology. 50: 354-362.

McColl, J.G., & Humphreys,F.R.(1967). Relationships between some nutritional factors and the distributions of Eucalyptus gummifera and Eucalyptus maculata. Ecology. 48: 766-771.

McElroy, C.T., & Rose, G. (1962). Reconnaissance geological survey: Ulladulla 1-mile military sheet, and southern part of Tianjara 1-mile military sheet. Geological Survey of New South Wales. New South Wales Department of Mines Bulletin. 17:77p.

Marks, G.C., Kassaby, F.Y., & Reynolds, S.T. (1972). Die-back in the mixed hardwood forests of eastern Victoria: a preliminary report. Australian Journal of Botany. 20: 141-154.

Marks, G.C., Kassaby, F.Y., & Fagg, P.C. (1973). Die-back tolerance in eucalypt species in relation to fertilization and soil populations of Phytophthora cinnamomi. Australian Journal of Botany. 21: 53-65.

Marx, D.H., & Bryan, W.C. (1969). Effect of soil bacteria on the mode of infection of pine roots by Phytophthora cinnamomi. Phytopathology. 59: 614-619.

Marx, D.H., & Davey, C.B. (1969 a). The influence of ectotrophic mycorrhizal fungi on the resistance of pine roots to pathogenic infections. III. Resistance of aseptically formed mycorrhizae to infection by Phytophthora cinnamomi. Phytopathology. 59: 549-558.

Marx, D.H., & Davey, C.B. (1969 b). The influence of ectotrophic mycorrhizal fungi on the resistance of pine roots to pathogenic infections. IV. Resistance of naturally occurring mycorrhizae to infections by Phytophthora cinnamomi. Phytopathology. 59: 559-565.

Marx, D.H., & Haasis, F.A. (1965). Production of aseptic sporangial formation in Phytophthora cinnamomi by metabolic diffusates of soil micro-organisms. Nature. 206: 673-674.

Mehrotra, R.S. (1970). Techniques for demonstrating accumulation of zoospores of Phytophthora species on roots in soil. Canadian Journal of Botany. 48: 879-882.

Menzel, R.G. (1954). Competitive uptake by plants of potassium, rubidium, cesuim, and calcium, strontium, barium from soils. Soil Science. 77: 419-425.

Menzies, J.D. (1970). Factors affecting plant pathogen population in soil. In ROOT DISEASES AND SOIL-BORNE PATHOGENS. Eds. T.A. Toussoun, R.V. Bega & P.E. Nelson. Berkeley, University of California Press. pp. 16-21.

Newhook, F.J. (1959). The association of Phytophthora spp. with mortality of Pinus radiata and other conifers. I. Symptoms and epidemiology in shelter belts. New Zealand Journal of Agricultural Research. 2: 808-843.

Newhook, F.J., & Podger, F.D. (1972). The role of Phytophthora cinnamomi in Australian and New Zealand forests. Annual Review of Phytopathology. 10: 299-326.

Northcote, K.H. (1966). Atlas of Australian Soils. Explanatory data for sheet 3, Sydney-Canberra-Bourke-Armidale area. Commonwealth Scientific and Industrial Research Organization, Australia. 107p.

Park, D. (1968). The ecology of terrestrial fungi. In The Fungi - An Advance Treatise. Vol. III - The fungal population. Eds.G.C. Ainsworth & A.S. Sussman. Academic Press, New York. pp.5-39.

Perley, A.F., & Page, O.T. (1969). Effect of calcium on activity of polygalacturonases induced in Fusarium roseum (Lk) emend. Snd. & Hans. Proceedings Canadian Phytopathological Society. 36: 18 (Abstract).

Podger, F.D. (1972). Phytophthora cinnamomi, a cause of lethal disease in indigenous plant communities in Western Australia. Phytopathology. 62: 972-981.

Podger, F.D., & Ashton, D.H. (1970). Phytophthora cinnamomi in dying vegetation on the Brisbane Ranges, Victoria. Australian Forest Research. 4(3): 33-36.

Podger, F.D., Doepel, R.F., & Zentmyer, G.A. (1965). Association of Phytophthora cinnamomi with a disease of Eucalyptus marginata forest in Western Australia. Plant Disease Reporter. 49: 943-947.

Pratt, B.H. (1971). Isolation of basidiomycetes from Australian eucalypt forest and assessment of their antagonism to Phytophthora cinnamomi. Transactions of the British Mycological Society. 56: 243-250.

Pratt, B.H., & Heather, W.A. (1971). Personal communication. Association of environmental factors with Phytophthora cinnamomi disease in native forest. Working Papers, Australian Plant Pathology Conference, Hobart. 3e 7-8.

Pratt, B.H., & Heather, W.A. (1972). Method for rapid differentiation of Phytophthora cinnamomi from other Phytophthora species isolated from soil by lupin baiting. Transactions of the British Mycological Society. 59: 87-96.

Pratt, B.H., & Heather, W.A. (1973 a). The origin and distribution of Phytophthora cinnamomi Rands in Australian native plant communities and the significance of its association with particular plant species. Australian Journal of Biological Science. 26: 559-573.

Pratt, B.H., & Heather, W.A. (1973 b). Recovery of potentially pathogenic Phytophthora and Pythium spp. from native vegetation in Australia. Australian Journal of Biological Science. 26: 575-583.

Pratt, B.H., Heather, W.A., & Shepherd, C.J. (1972). Transcontinental occurrence of A1 and A2 strains of Phytophthora cinnamomi in Australia. Australian Journal of Biological Science. 25: 1099-1100.

Pratt, B.H., Heather, W.A., & Shepherd, C.J. (1973). Recovery of Phytophthora cinnamomi from native vegetation in a remote area of New South Wales. Transactions of the British Mycological Society. 60: 197-204.

Pratt, B.H., Heather, W.A., Sedgley, J.H., & Shepherd, C.J. (1972 a). Phytophthora cinnamomi in natural vegetation in the A.C.T. Australian Plant Pathology Society Newsletter 1.

Pratt, B.H., Sedgley, J.H., Heather, W.A., & Shepherd, C.J. (1972 b). Oospore production in Phytophthora cinnamomi in the presence of Trichoderma koningii. Australian Journal of Biological Science. 25: 861-863.

Raney, W.A. (1965). Physical factors of the soil as they affect soil microorganisms. In ECOLOGY OF SOIL-BORNE PLANT PATHOGENS. Eds. K.F. Baker & W.C. Snyder. Berkeley, University of California Press. pp.115-119.

Rojas, E., & Tobias, J.M. (1965). Membrane model; association of inorganic cations with phospholipid monolayers. Biochimica et Biophysica Acta. 94: 394-404.

Shepherd, C.J., & Pratt, B.H. (1974). Temperature-growth relations and genetic diversity of A2 mating-type isolates of Phytophthora cinnamomi in Australia. Australian Journal of Botany. 22: (in press).

Shepherd, C.J., Pratt, B.H., & Taylor, P.A. (1974). Comparative morphology and behaviour of A1 and A2 compatibility type isolates of Phytophthora cinnamomi from Australian native plant communities. Australian Journal of Botany. 22: (in press).

Siegel, S. (1956). Nonparametric Statistics for the Behavioral Sciences. McGraw-Hill, New York, 312p.

Sokal, R.R., & Rohlf, F.J.(1970). Biometry. W.H. Freeman & Co, San Francisco. 776p.

Sommers, L.E., Harris, R.F., Dalton, F.N., & Gardner, W.R. (1970). Water potential relations of three root-infecting Phytophthora species. Phytopathology. 60: 932-934.

Thatcher, F.S.(1942). Further studies of osmotic and permeability relations in parasitism. Canadian Journal of Research. 20(c): 283-311.

Thomas, C.A. (1966). Calcium and water-insoluble pectic substances in safflower hypocotyls in relation to resistance to Phytophthora drechsleri. Phytopathology. 56: 985-986.

Trujillo, E.E., & Snyder, W.C. (1963). Uneven distribution of Fusarium oxysporum f. cubense in Honduras soils. Phytopathology. 53: 167-170.

Tsao, P.H. (1960). A serial dilution end point method for estimating disease potential of citrus Phytophthoras in soil. Phytopathology. 50: 717-724.

Turner, M.T., & Bateman, D.F. (1968). Maceration of plant tissues susceptible and resistant to soft-rot pathogens by enzymes from compatible host-pathogen combinations. Phytopathology. 58: 1509-1515.

Unbehaun, L.M., & Moore, L.D. (1970). Pectic enzymes associated with black root rot of tobacco. Phytopathology. 60: 304-308.

Veal, M. (personal communications). Pectic enzyme production by Phytophthora cinnamomi Rands. B.Sc. (Forestry) Honours Thesis. Australian National University, Canberra, 1971.

Waterhouse, G.M. (1963). Key to the species of Phytophthora de Bary. Mycological Papers. 92: 1-22.

Weste, G., Cooke, D., & Taylor, P. (1973). The invasion of native forest by Phytophthora cinnamomi. II. Post-infection vegetation patterns, regeneration, decline in inoculum, and attempted control. Australian Journal of Botany. 21: 13-29.

Weste, G.M., & Taylor, P. (1971). The invasion of native forest by Phytophthora cinnamomi. I. Brisbane Ranges, Victoria. Australian Journal of Botany. 19: 281-294.

Wills, W.H., & Moore, L.D. (1969). Calcium nutrition and black shank of tobacco. Phytopathology. 59: 346-351.

Wood, R.K.S. (1967). Physiological Plant Pathology. Blackwell Scientific Publications, Oxford. 570p.

Zentmyer, G.A.(1961). Chemotaxis of zoospores for root exudates. Science. 133: 1595-1596.

Zentmyer, G.A.(1965). Bacterial stimulation of sporangium production in Phytophthora cinnamomi. Science. 150: 1178-1179.

Zentmyer, G.A., & Thorn, W.A. (1967). Hosts of Phytophthora cinnamomi. California Avocado Society Yearbook No. 51.

APPENDIX I.

FURTHER STATISTICAL ANALYSIS OF ENVIRONMENTAL PARAMETERS NOT SIGNIFICANTLY ASSOCIATED WITH THE DISTRIBUTION OF P. CINNAMOMI IN SOIL

TABLE A. Sites positive for P. cinnamomi on basis of topography and ground cover

Topography	Ground cover			Total
	>50% litter	>50% litter + grasses	<50% litter	
Ridge	13/16 (12.7)[a]	1/2 (7.4)	9/12 (9.9)	23/30
Midslope	12/22 (22.4)	13/14 (13.1)	13/17 (17.5)	38/53
Gully	3/8 (5.9)	4/8 (3.5)	2/3 (4.6)	9/14
Total	28/41	18/24	24/32	70/97

[a] Expected frequency (from chi-square analysis)

Ground cover was found to be related to topography (X^2 = 13.09, d.f. = 4, p = <0.05), ground covers of >50% litter + grasses being confined largely to the gullies and lower slopes. A two-factor analysis of variance of the environmental parameters used in this and the following tables indicated no association between the presence of P. cinnamomi and each of the two parameters.

APPENDIX I. (Cont'd.)

TABLE B. Sites positive for P. cinnamomi on basis of distance from roads and ground cover

Distance from roads	Ground cover			Total
	>50% litter	>50% litter + grasses	<50% litter	
<20 metres	10/12 (13.5)	5/6 (7.9)	11/14 (10.6)	26/32
>20 <100 metres	13/19 (14.4)	4/5 (8.4)	9/10 (11.2)	26/34
>100 metres	5/10 (13.1)	9/13 (7.7)	4/8 (10.2)	18/31
Total	28/41	18/24	24/32	70/97

Ground cover was found to be related to distance from roads ($X^2 = 9.54$, d.f. = 4, p = 0.05), ground covers of >50% litter + grasses occurring in greater than expected frequencies in sites >100 metres from roads. However this may be largely associated with the location of roads along ridges (Table 2.3.2) and the relationship between ground cover and topography (Table A).

APPENDIX I. (Cont'd.)

TABLE C. Sites positive for P. cinnamomi on basis of species association and ground cover

Eucalyptus spp. association	Ground cover			Total
	>50% litter	>50% litter + grasses	<50% litter	
E. gummifera	10/13 (10.6)	2/4 (6.2)	7/8 (8.2)	19/25
E. maculata	12/19 (22.8)	13/14 (13.4)	15/21 (17.8)	40/54
E. saligna	6/9 (7.6)	3/6 (4.4)	2/3 (6.0)	11/18
Total	28/41	18/24	24/32	70/97

No relationship was found between Eucalyptus spp. association and ground cover (X^2 = 4.90, d.f. = 4, N.S.)

APPENDIX I. (Cont'd.)

TABLE D. Sites positive for P. cinnamomi on basis of distance from roads and soil moisture levels

Distance from roads	Soil moisture level			Total
	Dry	Intermediate	Moist	
<20 metres	12/15 (11.5)	6/8 (10.2)	8/9 (10.2)	26/32
>20 <100 metres	13/14 (12.3)	8/13 (10.9)	5/7 (10.9)	26/34
>100 metres	6/6 (11.2)	5/10 (9.9)	7/15(9.9)	18/31
Total	31/35	19/31	20/31	70/97

No relationship was found between soil moisture levels and distance from roads (X^2 = 8.73, d.f. = 4, N.S.)

TABLE E. Sites positive for P. cinnamomi on basis of species association and aspect

Eucalyptus spp. association	Aspect				Total
	0^o-90^o	90^o-180^o	180^o-270^o	270^o-360^o	
E. gummifera	6/8 (8.0)	1/2 (4.1)	7/9 (7.2)	5/6 (5.7)	19/25
E. maculata	14/18(17.2)	6/11 (8.9)	9/11(15.6)	11/14 (12.2)	40/54
E. saligna	3/5 (5.8)	3/3 (3.0)	4/8 (5.2)	1/2 (4.1)	11/18
Total	23/31	10/16	20/28	17/22	70/97

No relationship was found between Eucalyptus spp. association and aspect (X^2 = 1.40, d.f. = 2, N.S.)

APPENDIX I. (Cont'd.)

TABLE F. Sites positive for P. cinnamomi on basis of topography and aspect

Topography	Aspect				Total
	0^{o}–90^{o}	90^{o}–180^{o}	180^{o}–270^{o}	270^{o}–360^{o}	
Ridge	7/11 (9.6)	3/4 (5.0)	7/8 (8.7)	6/7 (6.8)	23/30
Midslope	14/17 (16.9)	5/9 (8.7)	10/15 (15.3)	9/12 (12.0)	38/53
Gully	2/3 (4.5)	2/3 (2.3)	3/5 (4.0)	2/3 (3.2)	9/14
Total	23/31	10/16	20/28	17/22	70/97

No relationship was found between topographic location of sampling sites and aspect (X^2 = 0.22, d.f. = 2, N.S.)

TABLE G. Sites positive for P. cinnamomi on basis of distance from roads and aspect

Distance from roads	Aspect				Total
	0^{o}–90^{o}	90^{o}–180^{o}	180^{o}–270^{o}	270^{o}–360^{o}	
< 20 metres	8/12 (10.2)	5/5 (5.3)	8/9 (9.2)	5/6 (7.3)	26/32
> 20 < 100 metres	10/12 (10.9)	3/5 (5.6)	8/10(9.8)	5/7 (7.7)	26/34
> 100 metres	5/7 (9.9)	2/6 (5.1)	4/9 (9.0)	7/9 (7.0)	18/31
Total	23/31	10/16	20/28	17/22	70/97

No relationship was found between distance of sampling sites from roads and aspect (X^2 = 2.33, d.f. = 6, N.S.).

APPENDIX II.

COMPOSITION OF NUTRIENT SOLUTIONS USED TO DETERMINE THE EFFECT OF CATIONS ON THE COLONIZATION OF LUPIN ROOTS BY P. CINNAMOMI

(a) Micronutrient stock solution

2.86 gm	H_3BO_3
1.81 gm	$MnCl_2.4H_2O$
0.11 gm	$ZnCl_2$
0.05 gm	$CuCl_2.2H_2O$
0.05 gm	$Ca(NO_3)_2.6H_2O$
0.025 gm	$NaMoO_4.2H_2O$

These chemicals were dissolved in 1 litre of glass-distilled water.

(b) Iron-EDTA stock solution

3.72 gm of EDTA and 2.49 gm of $FeSO_4.7H_2O$ were dissolved in 1 litre of glass-distilled water and stirred until the precipitate had dissolved.

1. Nutrient solutions for calcium treatment (quantities quoted per litre of final solution)

Each calcium treatment contained :-

1 M $MgSO_4$ (2 ml), 0.5 M KH_2PO_4 (2 ml), 1 M KNO_3 (5 ml), 2 M $NaNO_3$ (5 ml), micronutrients (1 ml), iron-EDTA sol. (1 ml).

APPENDIX II. (Cont'd.)

The calcium concentrations were adjusted by adding $CaCl_2.2H_2O$ in the following quantities:-

0.0369 gm (0.0003 M Ca), 0.1471 gm (0.001 M Ca),

0.2942 gm (0.002 M Ca), 0.4413 gm (0.003 M Ca),

0.5884 gm (0.004 M Ca), 0.7351 gm (0.005 M Ca) and

1.4702 gm (0.01 M Ca)

The solutions were made up to volume with glass-distilled water.

2. Nutrient solutions for magnesium treatment (quantities quoted per litre of final solution)

Each magnesium treatment contained:-

0.5 M KH_2PO_4 (2 ml), 1 M KNO_3 (5 ml), 1 M $Ca(NO_3)_2$ (4 ml),

2 M $NaNO_3$ (4 ml), 1 M Na_2SO_4 (1 ml), micronutrients (1 ml),

iron-EDTA sol. (1 ml).

The magnesium concentrations were adjusted by adding 1 M $MgCl_2$ in the following quantities:-

1 ml (0.001 M Mg), 5 ml (0.005 M Mg), 7.5 ml (0.0075 M Mg),

10 ml (0.01 M Mg) and 12.5 ml (0.0125 M Mg).

The solutions were made up to volume with glass-distilled water.

APPENDIX II. (Cont'd.)

3. Nutrient solutions for potassium treatment (quantities quoted per litre of final solution)

Each potassium treatment contained:-

1 M $MgSO_4$ (2 ml), 1 M $CaCl_2$ (5 ml), 1 M $Ca(NO_3)_2$ (5 ml),

2 M $NaNO_3$ (2.5 ml), micronutrients (1 ml), iron-EDTA sol. (1 ml).

The potassium concentrations were adjusted as follows:-

	nil K	0.003 M K	0.006 M K	0.05 M K	0.1 M K
1 M KCl	-	3 ml	5 ml	50 ml	100 ml
0.5 M KH_2PO_4	-	-	2 ml	2 ml	2 ml
0.5 M NaH_2PO_4	2 ml	2 ml	-	-	-

The solutions were made up to volume with glass-distilled water.

4. Nutrient solutions for calcium chelate treatment (quantities quoted per litre of final solution)

Each calcium chelate treatment contained:-

1 M $MgSO_4$ (2 ml), 0.5 M KH_2PO_4 (2 ml), 1 M KNO_3 (5 ml),

2 M $NaNO_3$ (5 ml), micronutrients (1 ml), iron-EDTA sol. (1 ml).

The calcium concentration (which was measured in each treatment by atomic absorption spectrophotometric analysis) was adjusted by adding a calcium chelate (Sequestren Na_2Ca containing 9.2% metallic calcium) in the following quantities:-

0.109 gm (0.0003 M Ca), 0.435 gm (0.0013 M Ca), 0.870 gm (0.0027 M Ca), 1.304 gm (0.0037 M Ca) and 1.740 gm (0.0050 M Ca).

The solutions were made up to volume with glass-distilled water.

APPENDIX II. (Cont'd.)

5. Nutrient solutions for aluminium treatment (quantities quoted per litre of final solution)

Each aluminium treatment contained:-

1 M $MgSO_4$ (2 ml), 0.5 M KH_2PO_4 (2 ml), 1 M KNO_3 (5 ml), 1 M $Ca(NO_3)_2$ (5 ml), micronutrients (1 ml), iron-EDTA sol. (1 ml).

The aluminium concentrations were adjusted by adding 0.5 M Al_2Cl_3 in the following quantities:-

0.5 ml (0.0005 M Al), 1 ml (0.001 M Al), 2 ml (0.002 M Al) and 3 ml (0.003 M Al).

The solutions were made up to volume with glass-distilled water.